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(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

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- as to the identity of the inventor (Rule 4.17(i)) for all designations
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(54) Title: METHODS OF DIAGNOSING AND TREATING SMALL INTESTINAL BACTERIAL OVERGROWTH (SIBO) AND SIBO-RELATED CONDITIONS

(57) Abstract: Disclosed is a method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject. SIBO-caused conditions include irritable bowel syndrome, fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, depression, impaired mentation; impaired memory, halitosis, tinnitus, sugar craving, autism, attention deficit/hyperactivity disorder, drug sensitivity, an autoimmune disease, and Crohn's disease. Also disclosed are a method of screening for the abnormally likely presence of SIBO in a human subject and a method of detecting SIBO in a human subject. A method of determining the relative severity of SIBO or a SIBO-caused condition in a human subject, in whom small intestinal bacterial overgrowth (SIBO) has been detected, is also disclosed.

WO 02/083926 A3

F18

INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 9/00

US CL : 426/71

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/2, 71

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| Y | US 5,776,524 A (REINHART) 07 July 1998 (07.07.1998), abstract, columns 1 and 2. | 1, 2 |
| X | COSTELLO et al. The Effect of an Elemental Diet on Stool Output in Irritable Bowel Syndrome. Proceedings of the Nutrition Society. 1994, Volume 53, Number 3, page 223A, see entire document. | 1, 2, 4, 5 |
| Y | MORISSE et al Effect of a Fructo-oligo-saccharides Compound in Rabbits Experimentally Infected with E-coli O.103., Journal of Applied Rabbit Research. 1992, Volume 15, pages 1137-1143, see entire document. | 1, 2 |
| A | YOUNG et al Colorectal Disorders: A Dietary Management Perspective. Asia Pacific Journal of Clinical Nutrition. 2000, Volume 9, (Supp.), pages S76-S82. | 1, 2, 4-7. |
| A | CUMMINGS et al. The Control and Consequences of Bacterial Fermentation in the Human Colon. Journal of Applied Bacteriology. 1991, Volume 70, pages 443-459. | 1, 2, 4-7 |
| A | SIMPSON, James W. Diet and Large intestinal Disease in Dogs and Cats. Journal of Nutrition. December 1998, Volume 128 (12 Supp.), pages 2717S-2722S. | 1, 2, 4-7 |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

| Special categories of cited documents: | |
|---|--|
| "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "E" earlier application or patent published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family |
| "P" document published prior to the international filing date but later than the priority date claimed | |

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INTERNATIONAL SEARCH REPORT

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1,2 and 4-7

Remark on Protest

☐
☒

The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-7, drawn to methods of treating small intestinal bacterial overgrowth (SIBO) in a subject by depriving the bacterial population of nutrients comprising causing the subject to consume a diet consisting essentially of nutrients that are at least partially predigested in the upper gastrointestinal tract.

Group II, claim(s) 1, and 8-20, drawn to methods of treating SIBO in a subject by depriving the bacterial population of nutrients comprising enhancing the digestion and/or absorption of the nutrients across the upper gastrointestinal tract.

Group III, claim(s) 21-24, drawn to methods of treating SIBO in a subject comprising introducing into the subject's small intestine's lumen a disinfectant composition in an amount sufficient to inhibit the growth of the bacteria.

Group IV, claim(s) 25-28, drawn to methods of treating SIBO in a subject comprising administering to the subject a pharmaceutical composition comprising a stabilizer of the mast cell membranes in the luminal wall of the small intestine.

Group V, claim(s) 29, drawn to a method of screening for the abnormally likely presence of SIBO in human subject by determining the concentration of one or more substances from the group consisting of serotonin, an unconjugated bile acid, and a folate.

Group VI, claim(s) 30-37, drawn to methods of detecting SIBO in a subject by detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in the gas exhaled by the subject after ingesting a controlled quantity of a substrate.

Group VII, claim(s) 38, drawn to methods of determining the severity of SIBO in a subject by detecting the relative level of intestinal permeability in a subject in whom SIBO has been detected.

Each of Groups I-VI comprise multiple subgroups of inventions. Therefore, if the applicant elects any of these Groups to be searched, the applicant must also elect one of the appropriate subgroups below.

For Group II, the applicant must elect one of the subgroups (II-a)-(II-m). The subgroups of Group II comprise the method of the Group, wherein the active agent is one of

- (II-a) an active lipid;
- (II-b) serotonin or a serotonin agonist;
- (II-c) a serotonin re-uptake inhibitor;
- (II-d) peptide YY;
- (II-e) calcitonin gene-related peptide;
- (II-f) adrenergic agonist;
- (II-g) opioid agonists;
- (II-h) a serotonin receptor antagonist;
- (II-i) a serotonin re-uptake inhibitor receptor antagonist;
- (II-j) a peptide YY receptor antagonist;
- (II-k) a calcitonin gene-related peptide receptor antagonist;
- (II-l) an adrenergic agonist receptor antagonist; and
- (II-m) an opioid agonist receptor antagonist.

For Group V, the applicant must elect one of the subgroups (V-a)-(V-c). The subgroups of Group V comprise the method of the Group, wherein the compound concentration detected is one of

- (V-a) serotonin;
- (V-b) one or more unconjugated bile acids; and
- (V-c) folate.

INTERNATIONAL SEARCH REPORT

PCT/US02/12034

For Group VI, the applicant must elect one of the subgroups (VI-a)-(VI-f). The subgroups of Group VI comprise the method of the Group, wherein the method used to detect the relative amounts of the gases is one of

- (VI-a) gas chromatography and/or by using a radiation detection system
- (VI-b) mass spectrometry;
- (VI-c) thin-layer chromatography;
- (VI-d) high pressure chromatography;
- (VI-e) using an electrochemical voltametric sensor; and
- (VI-f) using a polarographic cell.

For each of Groups I-III, and IV, the applicant must also elect one of the following subinventions of the respective Group of inventions. For Groups I-III, the applicant must elect one of groups (i)-(xvi). For Group IV, the applicant must elect one of groups (ii)-(xvi). The species are as follows, the method of the elected Group wherein the SIBO related condition is one of the diseases in a group consisting of:

- (i) irritable bowel syndrome;
- (ii) fibromyalgia;
- (iii) chronic pelvic pain syndrome;
- (iv) chronic fatigue syndrome;
- (v) depression;
- (vi) impaired mentation;
- (vii) impaired memory;
- (viii) halitosis;
- (ix) tinnitus;
- (x) sugar craving;
- (xi) autism;
- (xii) attention deficit/hyperactivity disorder;
- (xiii) drug sensitivity;
- (xiv) systemic lupus erythematosus;
- (xv) multiple sclerosis; and
- (xvi) Crohn's disease.

The inventions listed as Groups (IIa)-(II-m) do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: they each relate to a method of treating a disease wherein the method comprises the use of a different compound. As each of the methods uses a different compound, they methods have different modes of operation. They therefore share no common special technical feature.

The inventions listed as Groups (V-a)-(V-c) do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the methods measures the concentrations of a different compound in order to screen for a disorder. As the methods are screening for different compounds as the indicators of diseases, the methods have different modes of operation, and therefore share no common special technical feature.

The inventions listed as Groups (VI-a)-(VI-f) do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the methods detects SIBO in a subject through the use of a different process. As different processes are being used, the methods have different modes of operation, and therefore share no common special technical feature.

The inventions listed as Groups (i)-(xvi) do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the groups relates to a method relating to a different disease. As the methods relate to methods of treating different diseases, they are performing different functions, and achieving different effects, and therefore share no common special technical feature.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: although each of the methods is a method of treating SIBO, they each use a different process, and a different set of compounds. As the methods use different processes and compounds, they have different modes of operation. They therefore share no common special technical feature.

The inventions listed as Groups I-IV, Group V, Group VI, or Group VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the methods is performing a different function. As the methods are performing different functions, they share no common special technical feature.

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CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
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European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
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WO 02/083926 A2

METHODS OF DIAGNOSING AND TREATING SMALL INTESTINAL BACTERIAL OVERGROWTH (SIBO) AND SIBO-RELATED CONDITIONS

BACKGROUND OF THE INVENTION

5 Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

1. The Field of the Invention

10 This invention relates to the medical arts. It relates to a method of diagnosing and treating small intestinal bacterial overgrowth (SIBO), and conditions caused by SIBO.

2. Discussion of the Related Art

Small intestinal bacterial overgrowth (SIBO), also known as small bowel bacterial overgrowth (SBBO), is an abnormal condition in which aerobic and anaerobic enteric
15 bacteria from the colon proliferate in the small intestine, which is normally relatively free of bacterial contamination. SIBO is defined as greater than 10^6 CFU/mL small intestinal effluent (R.M. Donaldson, Jr., *Normal bacterial populations of the intestine and their relation to intestinal function*, N. Engl. J. Med. 270:938-45 [1964]). Typically, the symptoms include abdominal pain, bloating, gas and alteration in bowel habits, such as
20 constipation and diarrhea.

Irritable bowel syndrome, Crohn's disease, chronic fatigue syndrome, chronic pelvic pain syndrome, fibromyalgia, depression, attention deficit/hyperactivity disorder, autism, and autoimmune diseases, e.g., multiple sclerosis and systemic lupus erythematosus, are all clinical conditions of unclear etiology. No association has been made heretofore between
25 any of the afore-going diagnostic categories and SIBO.

Irritable bowel syndrome (IBS) is the most common of all gastrointestinal disorders, affecting 11-14% of adults and accounting for more than 50% of all patients with digestive complaints. (G. Triadafilopoulos *et al.*, *Bowel dysfunction in fibromyalgia*, Digestive Dis. Sci. 36(1):59-64 [1991]; W.G. Thompson, *Irritable Bowel syndrome: pathogenesis and
30 management*, Lancet 341:1569-72 [1993]). It is thought that only a minority of people with IBS actually seek medical treatment. Patients with IBS present with disparate symptoms, for

example, abdominal pain predominantly related to defecation, alternating diarrhea and constipation, abdominal distention, gas, and excessive mucus in the stool.

A number of possible causes for IBS have been proposed, but none has been fully accepted. (W.G. Thompson [1993]). These hypotheses included a fiber-poor Western diet,
5 intestinal motility malfunction, abnormal pain perception, abnormal psychology or behavior, or psychophysiological response to stress.

A high fiber diet increases stool bulk and shortens gut transit time. However the presence of IBS in non-Western countries, such as China and India, and the failure of dietary fiber supplements to treat IBS in double-blind clinical trials are inconsistent with the fiber
10 hypothesis for the causation of IBS. (W. Bi-zhen and P. Qi-Ying, *Functional bowel disorders in apparently healthy Chinese people*, Chin. J. Epidemiol. 9:345-49 [1988]; K.W. Heaton, *Role of dietary fibre in irritable bowel syndrome*. In: R.W. Read [ed.], *Irritable bowel syndrome*, Grune and Stratton, London, pp. 203-22 [1985]; W.G. Thompson *et al.*, *Functional bowel disorders and functional abdominal pain*, Gastroenterol. Int. 5:75-92
15 [1992]).

Those experiencing chronic IBS pain are often depressed and anxious. Treatment with tricyclic antidepressants has been used to raise the pain threshold of some IBS patients. (W.G. Thompson [1993]). Abreu *et al.* and Rabinovich *et al.* taught the use of corticotropin-releasing factor antagonists to relieve stress-related symptoms, including
20 depression and anxiety, in IBS, anorexia nervosa, and other disorders. (M.E. Abreu, *Corticotropin-releasing factor antagonism compounds*, U.S. Patent No. 5,063,245; A.K. Rabinovich *et al.*, *Benzoperimidine-carboxylic acids and derivatives thereof*, U.S. Patent No. 5,861,398). Becker *et al.* taught the use of serotonin antagonists to treat depression and anxiety associated with IBS and other conditions. (D.P. Becker *et al.*, *Meso-azacyclic aromatic acid amides and esters as serotonergic agents*, U.S. Patent No. 5,612,366).
25

Those with IBS symptoms have not been shown to have a different psychological or psychosocial make-up from the normal population. (W.E. Whitehead *et al.*, *Symptoms of psychologic distress associated with irritable bowel syndrome: comparison of community and medical clinic samples*, Gastroenterol. 95:709-14 [1988]). But many IBS patients appear
30 to perceive normal intestinal activity as painful. For example, IBS patients experience pain at lower volumes of rectal distention than normal or have a lower than normal threshold for perceiving migrating motor complex phase III activity. (W. E. Whitehead *et al.*, *Tolerance for rectosigmoid distension in irritable bowel syndrome*, Gastroenterol. 98:1187-92 [1990];

J.E. Kellow *et al.*, *Enhanced perception of physiological intestinal motility in the irritable bowel syndrome*, *Gastroenterol.* 101(6):1621-27 [1991]).

Bowel motility in IBS patients differs from normal controls in response to various stimuli such as drugs, hormones, food, and emotional stress. (D.G. Wangel and D.J. Deller, *Intestinal motility in man, III: mechanisms of constipation and diarrhea with particular reference to the irritable bowel*, *Gastroenterol.* 48:69-84 [1965]; R.F. Harvey and A.E. Read, *Effect of cholecystokinin on colon motility on and symptoms in patients with irritable bowel syndrome*, *Lancet* i:1-3 [1973]; R.M. Valori *et al.*, *Effects of different types of stress and prokinetic drugs on the control of the fasting motor complex in humans*, *Gastroenterol.* 90:1890-900 [1986]).

Evans *et al.* and Gorard and Farthing recognized that irritable bowel syndrome is frequently associated with disordered gastro-intestinal motility. (P.R. Evans *et al.*, *Gastroparesis and small bowel dysmotility in irritable bowel syndrome*, *Dig. Dis. Sci.* 42(10):2087-93 [1997]; DA. Gorard and M.J. Farthing, *Intestinal motor function in irritable bowel syndrome*, *Dig. Dis.* 12(2):72-84 [1994]). Treatment directed to bowel dysmotility in IBS includes the use of serotonin antagonists (D.P. Becker *et al.*, *Meso-azacyclic aromatic acid amides and esters as serotonergic agents*, U.S. Patent No. 5,612,366; M. Ohta *et al.*, *Method of treatment of intestinal diseases*, U.S. Patent No. 5,547,961) and cholecystokinin antagonists (Y. Sato *et al.*, *Benzodiazepine derivatives*, U.S. Patent No. 4,970,207; H. Kitajima *et al.*, *Thienylazole compound and thienotriazolodiazepine compound*, U.S. Patent No. 5,760,032). But colonic motility index, altered myoelectrical activity in the colon, and small intestinal dysmotility have not proven to be reliable diagnostic tools, because they are not IBS-specific. (W. G. Thompson [1993]).

Because there has been no known underlying cause for IBS, treatment of IBS has been primarily directed to symptoms of pain, constipation or diarrhea symptoms.

For example, administration of the polypeptide hormone relaxin, used to relax the involuntary muscles of the intestines, is a treatment taught to relieve the pain associated with IBS. (S.K. Yue, *Method of treating myofascial pain syndrome with relaxin*, U.S. Patent No. 5,863,552).

Borody *et al.* taught the use of a picosulfate-containing laxative preparation to treat constipation in IBS, small intestinal bacterial overgrowth, and acute or chronic bacterial bowel infections. (T.J. Borody *et al.*, *Picosulfate-containing preparation for colonic evacuation*, U.S. Patent No. 5,858,403). Barody also taught the use of an anti-inflammatory

agent to treat IBS. (T.J. Borody, *Treatment of non-inflammatory and non-infectious bowel disorders*, U.S. Patent No. 5,519,014). In addition, constipation in IBS has been treated with amidinourea compounds. (J. Yelnosky *et al.*, *Amidinoureas for treating irritable bowel syndrome*, U.S. Patent Nos. 4,701,457 and 4,611,011).

5 Kuhla *et al.* taught the use of triazinone compounds to relieve IBS symptoms of constipation, diarrhea, and abdominal pain. (D.E. Kuhla *et al.*, *Triazinones for treating irritable bowel syndrome*, U.S. Patent No. 4,562,188). And Kitazawa *et al.* taught the use of naphthyl- and phenyl- sulfonylalkanoic acid compounds to treat IBS symptoms. (M. Kitazawa *et al.*, *Naphthylsulfonylalkanoic acid compounds and pharmaceutical compositions thereof*, U.S. Patent No. 5,177,069; M. Kitazawa *et al.*, *Phenylsulfonylalkanoic acid compounds and pharmaceutical compositions thereof*, U.S. Patent No. 5,145,869). Day
10 taught an IBS treatment involving the administration of an anion-binding polymer and a hydrophilic polymer. (C.E. Day, *Method for treatment of irritable bowel syndrome*, U.S. Patent No. 5,380,522). And Borody *et al.* taught the use of salicylic acid derivatives to treat
15 IBS. (T.J. Borody *et al.*, *Treatment of non-inflammatory and non-infectious bowel disorders*, U.S. Patent No. 5,519,014).

 A probiotic approach to the treatment of IBS has also been tried. For example, Allen *et al.* described the use of a strain of *Enterococcus faecium* to alleviate symptoms. (W.D. Allen *et al.*, *Probiotic containing Enterococcus faecium strain NCIMB 40371*, U.S. Pat. No.
20 5,728,380 and *Probiotic*, U.S. Pat. No. 5,589,168). Borody taught a method of treating irritable bowel syndrome by at least partial removal of the existing intestinal microflora by lavage and replacement with a new bacterial community introduced by fecal inoculum from a disease-screened human donor or by a composition comprising *Bacteroides* and *Escherichia coli* species. (T.J. Borody, *Treatment of gastro-intestinal disorders with a fecal*
25 *composition or a composition of bacteroides and E. coli*, U.S. Pat. No. 5,443,826).

 Fibromyalgia (FM) is a syndrome of intense generalized pain and widespread local tenderness, usually associated with morning stiffness, fatigue, and sleep disturbances. (F. Wolfe, *Fibromyalgia: the clinical syndrome*, *Rheum. Dis. Clin. N. Amer.* 15(1):1-17 [1989]). Fibromyalgia is often associated with IBS (34-50% of FM cases) or other gastrointestinal
30 symptoms, Raynaud's phenomenon, headache, subjective swelling, paresthesias, psychological abnormality or functional disability, sometimes with overlapping symptoms of coexisting arthritis, lower back and cervical disorders, and tendonitis. Fibromyalgia

affects 1-5% of the population and is more prevalent among women than men. (G. Triadafilopoulos *et al.* [1991]).

As in IBS, a diagnosis of FM correlates with a decreased pain threshold among FM patients compared to non-patients. (F. Wolfe *et al.*, *Aspects of Fibromyalgia in the General Population: Sex, Pain Threshold, and Fibromyalgia Symptoms*, J. Rheumatol. 22:151-56 [1995]). But other conventional laboratory evaluations of FM patients are uniformly normal. (G. Triadafilopoulos *et al.* [1991]). The symptoms of FM patients are typically treated with anti-inflammatory agents and low dose tricyclic antidepressants. Administration of relaxin for involuntary muscle dysfunction is also a treatment taught to relieve the pain associated with fibromyalgia. (S.K. Yue, *Method of treating myofascial pain syndrome with relaxin*, U.S. Patent No. 5,863,552). However, there has been no known cause of FM to which diagnosis and/or treatment could be directed.

Chronic fatigue syndrome (CFS) affects more than a half million Americans. (P. H. Levine, *What we know about chronic fatigue syndrome and its relevance to the practicing physician*, Am. J. Med. 105(3A):100S-03S [1998]). Chronic fatigue syndrome is characterized by a sudden onset of persistent, debilitating fatigue and energy loss that lasts at least six months and cannot be attributed to other medical or psychiatric conditions; symptoms include headache, cognitive and behavioral impairment, sore throat, pain in lymph nodes and joints, and low grade fever. (M. Terman *et al.*, *Chronic Fatigue Syndrome and Seasonal Affective Disorder: Comorbidity, Diagnostic Overlap, and Implications for Treatment*, Am. J. Med. 105(3A):115S-24S [1998]). Depression and related symptoms are also common, including sleep disorders, anxiety, and worsening of premenstrual symptoms or other gynecological complications. (A.L. Komaroff and D. Buchwald, *Symptoms and signs of chronic fatigue syndrome*, Rev. Infect. Dis. 13:S8-S11 [1991]; B.L. Harlow *et al.*, *Reproductive correlates of chronic fatigue syndrome*, Am. J. Med. 105(3A):94S-99S [1998]).

Other physiologic abnormalities are also associated with CFS in many patients, including neurally-mediated hypotension, hypocortisolism, and immunologic dysregulation. (P.H. Levine [1998]). A subgroup of CFS patients complain of exacerbated mood state, diminished ability to work and difficulty awakening during winter months, reminiscent of seasonal affective disorder. (M. Terman *et al.* [1998]).

The etiology of CFS has been unknown, and the heterogeneity of CFS symptoms has precluded the use of any particular diagnostic laboratory test. (P.H. Levine [1998]). Symptomatic parallels have been suggested between CFS and a number of other disease

conditions, resulting from viral infection, toxic exposure, orthostatic hypotension, and stress, but none of these has been shown to have a causal role in CFS. (E.g., I.R. Bell *et al.*, *Illness from low levels of environmental chemicals: relevance to chronic fatigue syndrome and fibromyalgia*, Am. J. Med. 105(3A):74S-82S [1998]; R.L. Bruno *et al.*, *Parallels between post-polio fatigue and chronic fatigue syndrome: a common pathophysiology?*, Am. J. Med. 105(3A):66S-73S [1998]; R. Glaser and J.K. Kiecolt-Glaser, *Stress-associated immune modulation: relevance to viral infections and chronic fatigue syndrome*, Am. J. Med. 105(3A):35S-42S [1998]; P.C. Rowe and H. Calkins, *Neurally mediated hypotension and chronic fatigue syndrome*, Am. J. Med. 105(3A):15S-21S [1998]; L.A. Jason *et al.*, *Estimating the prevalence of chronic fatigue syndrome among nurses*, Am. J. Med. 105(3A):91S-93S [1998]). One study reported that there was no support for an etiological role in CFS of *Yersinia enterocolitica* infection. (C.M. Swanink *et al.*, *Yersinia enterocolitica and the chronic fatigue syndrome*, J. Infect. 36(3):269-72 [1998]). Accordingly, there has been no known cause to which diagnosis and/or treatment of CSF could be directed.

Consequently, the diagnosis and treatment of CFS have continued to be directed to symptoms, rather than to an underlying treatable cause. For example, the use of relaxin has been described for relaxing the involuntary muscles and thus relieve pain associated with CFS. (S.K. Yue, *Method of treating myofascial pain syndrome with relaxin*, U.S. Patent No. 5,863,552).

Attention deficit/hyperactivity disorder (ADHD) is a heterogeneous behavioral disorder of unknown etiology that always appears first in childhood, affecting 3-20 % of elementary school-age children, and continues to affect up to 3% of adults. (Reviewed in L.L. Greenhill, *Diagnosing attention deficit/hyperactivity disorder in children*, J. Clin. Psychiatry 59 Suppl 7:31-41 [1998]). Those affected with ADHD symptoms typically exhibit inattentiveness and distractability (AD type), hyperactive and impulsive behavior (HI type), or a combination of these, to a degree that impairs normal functioning and is often socially disruptive. (M.L. Wolraich *et al.*, *Examination of DSM-IV criteria for attention deficit/hyperactivity disorder in a county-wide sample*, J. Dev. Behav. Pediatr. 19(3):162-68 [1998]; J.J. Hudziak *et al.*, *Latent class and factor analysis of DSM-IV ADHD: a twin study of female adolescents*, J. Am. Acad. Child Adolesc. Psychiatry 37(8):848-57 [1998]). Often prescribed are central nervous system stimulants, tricyclic antidepressants, antihypertensives, analgesics, or antimanic drugs, but there has been no known cause of ADHD to which diagnosis and/or treatment could be directed. (S.C. Schneider and G. Tan, *Attention*

deficit/hyperactivity disorder. In pursuit of diagnostic accuracy, Postgrad. Med. 101(4):231-2, 235-40 [1997]; W.J. Barbaresi, *Primary-care approach to the diagnosis and management of attention deficit/hyperactivity disorder*, Mayo Clin. Proc. 71(5):463-71 [1996]).

There has also been no known cause for autoimmune diseases, including multiple sclerosis and systemic lupus erythematosus. Multiple sclerosis (MS) is a neurologic disease that primarily strikes teens and young adults under 35 years. Affecting 350,000 Americans, MS is the most frequent cause of neurologic disability except for traumatic injuries; MS affects twice as many females compared to males. (S.L. Hauser, *Multiple Sclerosis and other demyelinating diseases* In: *Harrison's Principles of Internal Medicine*, 13th ed., K.J. Isselbacher *et al.* (eds.), McGraw-Hill, pp.2287-95 [1994]). The disease is characterized by chronic inflammation, scarring, and selective destruction of the myelin sheath around neural axons of the central nervous system, and is thought to be caused by autoimmune responses.

A treatment for MS taught by Weiner *et al.* is related to oral administration of autoantigens to the patient to suppress the autoimmune response by eliciting suppressor T-cells specific for myelin basic protein (MBP). There are no specific diagnostic tests for MS; diagnosis is based on clinical recognition of destructive patterns of central nervous system injury that are produced by the disease. (S.L. Hauser [1994]) Nerve damage may be mediated by cytokines, especially TNF- α , which has been found to be selectively toxic to myelin and to oligodendrocytes in vitro. Elevated levels of TNF- α and IL-2 were measured in MS patients. (J.L. Trotter *et al.*, *Serum cytokine levels in chronic progressive multiple sclerosis: interleukin-2 levels parallel tumor necrosis factor-alpha levels*, J. Neuroimmunol. 33(1):29-36 [1991]; H.L. Weiner *et al.*, *Treatment of multiple sclerosis by oral administration of autoantigens*, U.S. Patent No. 5,869,054). Another treatment for MS involves the administration of a vitamin D compound. (H.F. DeLuca *et al.*, *Multiple sclerosis treatment*, U.S. Patent No. 5,716,946). However, there has been no known cause of MS to which diagnosis and/or treatment could be directed.

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by deposition in tissues of autoantibodies and immune complexes leading to tissue injury (B.L. Kotzin, *Systemic lupus erythematosus*, Cell 85:303-06 [1996]). In contrast to autoimmune diseases such as MS and type 1 diabetes mellitus, SLE potentially involves multiple organ systems directly, and its clinical manifestations are diverse and variable. (Reviewed by B.L. Kotzin and J.R. O'Dell, *Systemic lupus erythematosus*, In: *Samler's Immunologic Diseases*, 5th ed., M.M. Frank *et al.*, eds., Little Brown & Co.,

Boston, pp. 667-97 [1995]). For example, some patients may demonstrate primarily skin rash and joint pain, show spontaneous remissions, and require little medication. At the other end of the spectrum are patients who demonstrate severe and progressive kidney involvement that requires therapy with high doses of steroids and cytotoxic drugs such as cyclophosphamide. (B.L. Kotzin [1996]).

The serological hallmark of SLE, and the primary diagnostic test available, is elevated serum levels of IgG antibodies to constituents of the cell nucleus, such as double-stranded DNA (dsDNA), single-stranded DNA (ss-DNA), and chromatin. Among these autoantibodies, IgG anti-dsDNA antibodies play a major role in the development of lupus glomerulonephritis (GN). (B.H. Hahn and B. Tsao, *Antibodies to DNA*, In: *Dubois Lupus Erythematosus*, 4th ed., D.J. Wallace and B. Hahn, eds., Lea and Febiger, Philadelphia, pp. 195-201 [1993]; Ohnishi *et al.*, *Comparison of pathogenic and nonpathogenic murine antibodies to DNA: Antigen binding and structural characteristics*, *Int. Immunol.* 6:817-30 [1994]). Glomerulonephritis is a serious condition in which the capillary walls of the kidney's blood purifying glomeruli become thickened by accretions on the epithelial side of glomerular basement membranes. The disease is often chronic and progressive and may lead to eventual renal failure.

The mechanisms by which autoantibodies are induced in these autoimmune diseases remains unclear. As there has been no known cause of SLE, to which diagnosis and/or treatment could be directed, treatment has been directed to suppressing immune responses, for example with macrolide antibiotics, rather than to an underlying cause. (E.g., Hitoshi *et al.*, *Immunosuppressive agent*, U.S. Pat. No. 4,843,092).

Another disorder for which immunosuppression has been tried is Crohn's disease. Crohn's disease symptoms include intestinal inflammation and the development of intestinal stenosis and fistulas; neuropathy often accompanies these symptoms. Anti-inflammatory drugs, such as 5-aminosalicylates (e.g., mesalamine) or corticosteroids, are typically prescribed, but are not always effective. (Reviewed in V.A. Botoman *et al.*, *Management of Inflammatory Bowel Disease*, *Am. Fam. Physician* 57(1):57-68 [1998]). Immunosuppression with cyclosporine is sometimes beneficial for patients resistant to or intolerant of corticosteroids. (J. Brynskov *et al.*, *A placebo-controlled, double-blind, randomized trial of cyclosporine therapy in active chronic Crohn's disease*, *N. Engl. J. Med.* 321(13):845-50 [1989]).

Nevertheless, surgical correction is eventually required in 90% of patients; 50% undergo colonic resection. (K. Leiper *et al.*, *Adjuvant post-operative therapy*, Baillieres Clin. Gastroenterol. 12(1):179-99 [1998]; F. Makowiec *et al.*, *Long-term follow-up after resectional surgery in patients with Crohn's disease involving the colon*, Z. Gastroenterol. 36(8):619-24 [1998]). The recurrence rate after surgery is high, with 50% requiring further surgery within 5 years. (K. Leiper *et al.* [1998]; M. Besnard *et al.*, *Postoperative outcome of Crohn's disease in 30 children*, Gut 43(5):634-38 [1998]).

One hypothesis for the etiology of Crohn's disease is that a failure of the intestinal mucosal barrier, possibly resulting from genetic susceptibilities and environmental factors (e.g., smoking), exposes the immune system to antigens from the intestinal lumen including bacterial and food antigens (e.g., Söderholm *et al.*, *Epithelial permeability to proteins in the non-inflamed ileum of Crohn's disease?*, Gastroenterol. 117:65-72 [1999]; D. Hollander *et al.*, *Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor*, Ann. Intern. Med. 105:883-85 [1986]; D. Hollander, *The intestinal permeability barrier. A hypothesis to its involvement in Crohn's disease*, Scand. J. Gastroenterol. 27:721-26 [1992]). Another hypothesis is that persistent intestinal infection by pathogens such as *Mycobacterium paratuberculosis*, *Listeria monocytogenes*, abnormal *Escherichia coli*, or paramyxovirus, stimulates the immune response; or alternatively, symptoms result from a dysregulated immune response to ubiquitous antigens, such as normal intestinal microflora and the metabolites and toxins they produce. (R.B. Sartor, *Pathogenesis and Immune Mechanisms of Chronic Inflammatory Bowel Diseases*, Am. J. Gastroenterol. 92(12):5S-11S [1997]). The presence of IgA and IgG anti-*Saccharomyces cerevisiae* antibodies (ASCA) in the serum was found to be highly diagnostic of pediatric Crohn's disease. (F.M. Ruemmele *et al.*, *Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease*, Gastroenterol. 115(4):822-29 [1998]; E.J. Hoffenberg *et al.*, *Serologic testing for inflammatory bowel disease*, J. Pediatr. 134(4):447-52 [1999]).

In Crohn's disease, a dysregulated immune response is skewed toward cell-mediated immunopathology. (S.I. Murch, *Local and systemic effects of macrophage cytokines in intestinal inflammation*, Nutrition 14:780-83 [1998]). But immunosuppressive drugs, such as cyclosporine, tacrolimus, and mesalamine have been used to treat corticosteroid-resistant cases of Crohn's disease with mixed success. (J. Brynskov *et al.* [1989]; K. Fellerman *et al.*, *Steroid-unresponsive acute attacks of inflammatory bowel disease: immunomodulation by tacrolimus [FK506]*, Am. J. Gastroenterol. 93(10):1860-66 [1998]). An abnormal increase

in colonic permeability is also seen in patients with Crohn's disease. (Vermeire S. *et al.*, *Anti-Saccharomyces cerevisiae antibodies (ASCA), phenotypes of IBD, and intestinal permeability: a study in IBD families*, *Inflamm Bowel Dis.* 7(1):8-15 [2001]).

Recent efforts to develop diagnostic and treatment tools against Crohn's disease have focused on the central role of cytokines. (S. Schreiber, *Experimental immunomodulatory therapy of inflammatory bowel disease*, *Neth. J. Med.* 53(6):S24-31 [1998]; R.A. van Hogezaand and H.W. Verspaget, *The future role of anti-tumour necrosis factor-alpha products in the treatment of Crohn's disease*, *Drugs* 56(3):299-305 [1998]). Cytokines are small secreted proteins or factors (5 to 20 kD) that have specific effects on cell-to-cell interactions, intercellular communication, or the behavior of other cells. Cytokines are produced by lymphocytes, especially T_H1 and T_H2 lymphocytes, monocytes, intestinal macrophages, granulocytes, epithelial cells, and fibroblasts. (Reviewed in G. Rogler and T. Andus, *Cytokines in inflammatory bowel disease*, *World J. Surg.* 22(4):382-89 [1998]; H.F. Galley and N.R. Webster, *The immuno-inflammatory cascade*, *Br. J. Anaesth.* 77:11-16 [1996]). Some cytokines are pro-inflammatory (e.g., tumor necrosis factor [TNF]- α , interleukin [IL]-1(α and β), IL-6, IL-8, IL-12, or leukemia inhibitory factor [LIF]); others are anti-inflammatory (e.g., IL-1 receptor antagonist [IL-1ra], IL-4, IL-10, IL-11, and transforming growth factor [TGF]- β). However, there may be overlap and functional redundancy in their effects under certain inflammatory conditions.

In active cases of Crohn's disease, elevated concentrations of TNF- α and IL-6 are secreted into the blood circulation, and TNF- α , IL-1, IL-6, and IL-8 are produced in excess locally by mucosal cells. (Id.; K. Funakoshi *et al.*, *Spectrum of cytokine gene expression in intestinal mucosal lesions of Crohn's disease and ulcerative colitis*, *Digestion* 59(1):73-78 [1998]). These cytokines can have far-ranging effects on physiological systems including bone development, hematopoiesis, and liver, thyroid, and neuropsychiatric function. Also, an imbalance of the IL-1 β /IL-1ra ratio, in favor of pro-inflammatory IL-1 β , has been observed in patients with Crohn's disease. (G. Rogler and T. Andus [1998]; T. Saiki *et al.*, *Detection of pro- and anti-inflammatory cytokines in stools of patients with inflammatory bowel disease*, *Scand. J. Gastroenterol.* 33(6):616-22 [1998]; S. Dionne *et al.*, *Colonic explant production of IL-1 and its receptor antagonist is imbalanced in inflammatory bowel disease (IBD)*, *Clin. Exp. Immunol.* 112(3):435-42 [1998]; But see S. Kuboyama, *Increased circulating levels of interleukin-1 receptor antagonist in patients with inflammatory bowel disease*, *Kurume Med. J.* 45(1):33-37 [1998]). One study suggested that cytokine profiles

in stool samples could be a useful diagnostic tool for *Crohn's* disease. (T. Saiki *et al.* [1998]).

Treatments that have been proposed for *Crohn's* disease include the use of various cytokine antagonists (e.g., IL-1ra), inhibitors (e.g., of IL-1 β converting enzyme and antioxidants) and anti-cytokine antibodies. (G. Rogler and T. Andus [1998]; R.A. van Hogezaand and H.W. Verspaget [1998]; J.M. Reimund *et al.*, *Antioxidants inhibit the in vitro production of inflammatory cytokines in Crohn's disease and ulcerative colitis*, Eur. J. Clin. Invest. 28(2):145-50 [1998]; N. Lugering *et al.*, *Current concept of the role of monocytes/macrophages in inflammatory bowel disease balance of pro-inflammatory and immunosuppressive mediators*, Ital. J. Gastroenterol. Hepatol. 30(3):338-44 [1998]; M.E. McAlindon *et al.*, *Expression of interleukin 1 beta and interleukin 1 beta converting enzyme by intestinal macrophages in health and inflammatory bowel disease*, Gut 42(2):214-19 [1998]). In particular, monoclonal antibodies against TNF- α have been tried with some success in the treatment of *Crohn's* disease. (S.R. Targan *et al.*, *A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease*. *Crohn's Disease cA2 Study Group*, N. Engl. J. Med. 337(15):1029-35 [1997]; W.A. Stack *et al.*, *Randomised controlled trial of CDP571 antibody to tumour necrosis factor-alpha in Crohn's disease*, Lancet 349(9051):521-24 [1997]; H.M. van Dullemen *et al.*, *Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2)*, Gastroenterol. 109(1):129-35 [1995]).

Another approach to the treatment of *Crohn's* disease has focused on at least partially eradicating the bacterial community that may be triggering the inflammatory response and replacing it with a non-pathogenic community. For example, McCann *et al.* (McCann *et al.*, *Method for treatment of idiopathic inflammatory bowel disease*, U.S. Pat. No. 5,599,795) disclosed a method for the prevention and treatment of *Crohn's* disease in human patients.

Their method was directed to sterilizing the intestinal tract with at least one antibiotic and at least one anti-fungal agent to kill off the existing flora and replacing them with different, select, well-characterized bacteria taken from normal humans. Borody taught a method of treating *Crohn's* disease by at least partial removal of the existing intestinal microflora by lavage and replacement with a new bacterial community introduced by fecal inoculum from a disease-screened human donor or by a composition comprising *Bacteroides* and *Escherichia coli* species. (T.J. Barody, *Treatment of gastro-intestinal disorders with a fecal composition or a composition of bacteroides and E. coli*, U.S. Pat. No. 5,443,826). However,

there has been no known cause of *Crohn's* disease to which diagnosis and/or treatment could be directed.

Pain is a common symptom associated with irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, chronic pelvic pain syndrome, depression, ADHD, autoimmune diseases, and *Crohn's* disease. While the experience of pain is intertwined with a person's emotions, memory, culture, and psychosocial situation (D.A. Drossman and W.G. Thompson, *Irritable bowel syndrome: a graduated, multicomponent treatment approach*, Ann. Intern. Med. 116:1009-16 [1992]), evidence shows that certain cytokine mediated-immune responses can influence the perception of pain. Cytokines can be released in response to a variety of irritants and can modulate the perception of pain. For example, exposure of human bronchial epithelial cells to irritants, including acidic pH, results in a receptor-mediated release of inflammatory cytokines IL-6, IL-8, and TNF- α . (B. Veronesi *et al.*, *Particulate Matter initiates inflammatory cytokine release by activation of capsaicin and acid receptors in a human bronchial epithelial cell line*, Toxicol. Appl. Pharmacol. 154:106-15 [1999]). Irritant receptors on cell surfaces, e.g., receptors sensitive to noxious stimuli, such as capsaicin and pH, mediate the release of cytokines and also mediate the release of neuropeptides from sensory nerve fibers, which is known to result in a neurogenic inflammatory processes and hyperalgesia (excessive sensitivity to pain). (Id.; R.O.P. de Campos *et al.*, *Systemic treatment with Mycobacterium bovis bacillus calmett-guerin (BCG) potentiates kinin B₁ receptor agonist-induced nociception and oedema formation in the formalin test in mice*, Neuropeptides 32(5):393-403 [1998]).

The perception of pain, is also influenced by the mediation of kinin B₁ and B₂ receptors, which bind peptides called kinins, e.g., the nonapeptide bradykinin or the decapeptide kallidin (lysyl bradykinin). While the precise mechanism of action is unknown, kinins cause the release of other pro-inflammatory and hyperalgesic mediators such as neuropeptides. Cytokines IL-1(α and β), IL-2, IL-6, and TNF- α are thought to activate kinin B₁ receptor, and thus can contribute to enhanced perception of pain. (R.O.P. de Campos *et al.* [1998]. The endotoxin of *Escherichia coli* significantly activated kinin B₁ receptor-mediated neurogenic and inflammatory pain responses in animals. (M.M. Campos *et al.*, *Expression of B₁ kinin receptors mediating paw oedema formalin-induced nociception. Modulation by glucocorticoids*, Can. J. Physiol. Pharmacol. 73:812-19 [1995]).

It has also been shown that IL-1 β , IL-6, and TNF- α , administered to the mammalian brain, can modulate pain perception via prostaglandin-dependent processes. (T. Hori *et al.*,

Pain modulatory actions of cytokines and prostaglandin E₂ in the Brain, Ann. N.Y. Acad. Sci. 840:269-81 [1998]). Granulocytes, which accumulate in nearly all forms of inflammation, are non-specific amplifiers and effectors of specific immune responses, and they can also modulate the perception of pain. Neutrophils, a type of granulocyte cell, are known to accumulate in response to IL-1 β , and neutrophil accumulation plays a crucial positive role in the development of nerve growth factor (NGF)-induced hyperalgesia. (G. Bennett *et al.*, *Nerve growth factor induced hyperalgesia in the rat hind paw is dependent on circulating neutrophils*, Pain 77(3):315-22 [1998]; see also E. Feher *et al.*, *Direct morphological evidence of neuroimmunomodulation in colonic mucosa of patients with Crohn's disease*, Neuroimmunomodulation 4(5-6):250-57 [1997]).

Visceral hyperalgesia, or pain hypersensitivity, is a common clinical observation in small intestinal bacterial overgrowth (SIBO), Crohn's disease, chronic pelvic pain syndrome, and irritable bowel syndrome (IBS). As many as 60% of subjects with IBS have reduced sensory thresholds for rectal distension compared to normal subjects. (H. Mertz *et al.*, *Altered rectal perception is a biological marker of patients with the irritable bowel syndrome*, Gastroenterol. 109:40-52 [1995]). While the experience of pain is intertwined with a person's emotions, memory, culture, and psychosocial situation (D.A. Drossman and W.G. Thompson, *Irritable bowel syndrome: a graduated, multicomponent treatment approach*, Ann. Intern. Med. 116:1009-16 [1992]) and the etiology for this hyperalgesia has remained elusive, evidence shows that certain cytokine mediated-immune responses can influence the perception of pain. Cytokines, including IL-1(α and β), IL-2, IL-6, and TNF- α , can be released in response to a variety of irritants and can modulate the perception of pain, possibly through the mediation of kinin B₁ and/or B₂ receptors (see, M.M. Campos *et al.*, *Expression of B₁ kinin receptors mediating paw oedema formalin-induced nociception. Modulation by glucocorticoids*, Can. J. Physiol. Pharmacol. 73:812-19 [1995]; R.O.P. de Campos *et al.*, *Systemic treatment with Mycobacterium bovis bacillus calmett-guerin (BCG) potentiates kinin B₁ receptor agonist-induced nociception and oedema formation in the formalin test in mice*, Neuropeptides 32(5):393-403 [1998]). Cytokine and neuropeptide levels are altered in IBS. An increase in substance P (neuropeptide)-sensitive nerve endings has been observed in subjects with IBS. (X. Pang *et al.*, *Mast cell substance P-positive nerve involvement in a patient with both irritable bowel syndrome and interstitial cystitis*, Urology 47:436-38 [1996]). It has also been hypothesized that there is a sensitization of afferent pathways in IBS. (E.A. Mayer *et al.*, *Basic and clinical aspects of visceral hyperalgesia*, Gastroenterol

1994;107:271-93 [1994]; L. Bueno *et al.*, *Mediators and pharmacology of visceral sensitivity: from basic to clinical investigations*, Gastroenterol. 112:1714-43 [1997]).

Fibromyalgia, typically involving global musculoskeletal and/or cutaneous pain, is, by definition, a hyperalgesic state since the American College of Rheumatology defines
5 fibromyalgia as a history of global pain in the setting of 11 out of 18 predefined tender points. (F. Wolfe *et al.*, *The American College of Rheumatology 1990 criteria for the classification of fibromyalgia*, Arthritis Rheum. 33:160-72 [1990]). Evidence implies that the hyperalgesia of fibromyalgia is not simply trigger point-related but rather a global hyperalgesia. (L. Vecchiet *et al.*, *Comparative sensory evaluation of parietal tissues in
10 painful and nonpainful areas in fibromyalgia and myofascial pain syndrome*, In: Gebhart GF, Hammond DL, Jensen TS, editors, *Progress in Pain Research and Management*, Vol. 2, Seattle: IASP Press, pp.177-85 [1994]; J. Sorensen *et al.*, *Hyperexcitability in fibromyalgia*, J. Rheumatol. 25:152-55 [1998]).

Cytokine and neuropeptide levels are altered in IBS, fibromyalgia, and Crohn's
15 disease. It has been shown that levels of substance P, a neuropeptide associated with nociception, are elevated in the cerebrospinal fluid of subjects with fibromyalgia. (H. Vaeroy *et al.*, *Elevated CSF levels of substance P and high incidence of Raynaud's phenomenon in patients with fibromyalgia: new features for diagnosis*, Pain 32:21-26 [1988]; I.J. Russell *et al.*, *Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia
20 syndrome*, Arthritis Rheum. 37:1593-1601 [1994]). And an increase in substance P-sensitive nerve endings has been observed in subjects with IBS and Crohn's disease. (X. Pang *et al.*, *Mast cell substance P-positive nerve involvement in a patient with both irritable bowel syndrome and interstitial cystitis*, Urology 47:436-38 [1996]; (C.R. Mantyh *et al.*, *Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high
25 concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease*, Proc. Natl. Acad. Sci. 85:3235-39 [1988]; S. Mazumdar and K.M. Das, *Immunocytochemical localization of vasoactive intestinal peptide and substance P in the colon from normal subjects and patients with inflammatory bowel disease*, Am. J. Gastrol. 87:176-81 [1992]; C.R. Mantyh *et al.*,
30 *Differential expression of substance P receptors in patients with Crohn's disease and ulcerative colitis*, Gastroenterol. 1995;109:850-60 [1995]).

Patients with chronic pelvic pain are usually evaluated and treated by gynecologists, gastroenterologists, urologists, and internists, but in many patients with chronic pelvic pain

the examination and work-up remain unrevealing, and no specific cause of the pain, such as endometriosis, can be identified. In these cases the patient is commonly said to be suffering from a "chronic pelvic pain syndrome." Once the diagnosis of chronic pelvic pain is made, treatment is typically directed to symptomatic pain management, rather than to an underlying
5 cause. (Wesselmann U, Czakanski PP, *Pelvic pain: a chronic visceral pain syndrome*, Curr. Pain Headache Rep. 5(1):13-9 [2001]).

Mental functioning and feelings of fatigue or depression can also be influenced by immune responses. Peripherally released pro-inflammatory cytokines, such as IL-1, IL-6 and TNF- α , act on brain cellular targets and have been shown to depress spontaneous and learned
10 behavior in animals; the vagus nerve has been shown to mediate the transmissions of the immune message to the brain, resulting in production of pro-inflammatory cytokines centrally in the brain. (R. Dantzer *et al.*, *Cytokines and sickness behavior*, Ann. N.Y. Acad. Sci. 840:586-90 [1998]). In addition, there is bidirectional interplay between neurotransmitters and the immune system; lymphocytes and macrophages bear surface
15 receptors for the stress hormone corticotrophin releasing hormone (CRH), and they respond to CRH by enhanced lymphocyte proliferation and feedback upregulation of hypothalamic CRH production. (S.H. Murch [1998]).

Pituitary production of proopiomelanocortins, such as endorphins and enkephalins, is upregulated by IL-1 and IL-2, possibly mediated by CRH, and lymphocytes and
20 macrophages recognize these endogenous opiates via surface receptors. (S.H. Murch [1998]). Lymphocytes (T_H2) and macrophages also produce and process enkephalin to an active form. Macrophage-derived cytokines, such as TNF- α , IL-1, and IL-6, are known to modulate neurotransmitter release and to affect overall neural activity; cytokines can induce classic illness behavior such as somnolence, apathy, depression, irritability, confusion, poor
25 memory, impaired mental concentration, fever and anorexia.

While immunological responses of various severities can lead to symptoms characteristic of irritable bowel syndrome, fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, impaired mentation and/or memory, depression, autism, ADHD, autoimmune diseases, and Crohn's disease, there has been a definite need to determine a
30 causal factor, for each of these diagnostic categories, to which diagnostic testing and treatment can be directed effectively.

SIBO has, until recently, mostly been suspected in subjects with significant malabsorptive sequelae. Most of the described cases of SIBO involve anatomic alterations

such as physical obstruction (E.A. Deitch *et al.*, *Obstructed intestine as a reservoir for systemic infection*, Am. J. Surg. 159:394 [1990]), surgical changes (e.g., L.K. Enander *et al.*, *The aerobic and anaerobic microflora of the gastric remnant more than 15 years after Billroth II resection*, Scand. J. Gastroenterol. 17:715-20 [1982]), direct communication of the small intestine with colonic contents such as fistulae (O. Bergesen *et al.*, *Is vitamin B12 malabsorption in bile fistula rats due to bacterial overgrowth? A study of bacterial metabolic activity in the small bowel*, Scand. J. Gastroenterol. 23:471-6 [1988]) and ileocecal valve dysfunction (surgical or otherwise) (W.O. Griffin, Jr, *et al.*, *Prevention of small bowel contamination by ileocecal valve*, S. Med. J. 64: 1056-8 [1971]; P. Rutgeerts *et al.*, *Ileal dysfunction and bacterial overgrowth in patients with Crohn's disease*, Eur. J. Clin. Invest. 11:199-206 [1981]). Less commonly, SIBO has been associated with chronic pancreatitis (E. Trespi and A. Ferrieri, *Intestinal bacterial overgrowth during chronic pancreatitis*, Curr. Med. Res. Opin. 15:47-52 [1999]), hypochlorhydria (e.g., S.P. Pereira *et al.*, *Drug-induced hypochlorhydria causes high duodenal bacterial counts in the elderly*, Aliment. Pharmacol. Ther. 12:99-104 [1998]), and immunodeficiency (C. Pignata *et al.*, *Jejunal bacterial overgrowth and intestinal permeability in children with immunodeficiency syndromes*, Gut 31:879-82 [1990]; G.M. Smith *et al.*, *Small intestinal bacterial overgrowth in patients with chronic lymphocytic leukemia*, J. Clin. Pathol. 43:57-9 [1990]).

SIBO has been associated with infections of the abdominal cavity in cases of alcoholic cirrhosis. (F. Casafont Morencos *et al.*, *Small bowel bacterial overgrowth in patients with alcoholic cirrhosis*, Dig. Dis. Sci. 40(6):1252-1256 [1995]; J. Chesta *et al.*, *Abnormalities in proximal small bowel motility in patients with cirrhosis*, Hepatology 17(5):828-32 [1993]; C.S. Chang *et al.*, *Small intestine dysmotility and bacterial overgrowth in cirrhotic patients with spontaneous bacterial peritonitis*, Hepatology 28(5):1187-90 [1998]). SIBO has also been associated with symptoms of chronic diarrhea, anorexia or nausea in elderly patients, and the prevalence of overgrowth in subjects over 75 years old is reported to be as high as 79% even in the absence of clinically evident clues of overgrowth or achlorhydria. (S.M. Riordan *et al.*, *Small intestinal bacterial overgrowth in the symptomatic elderly*, Am. J. Gastroenterol. 92(1):47-51 [1997]). SIBO is also associated with chronic digestive symptoms in children, especially infants under two years of age (D. De Boissieu *et al.*, *Small-bowel bacterial overgrowth in children with chronic digestive diarrhea, abdominal pain, or both*, J. Pediatr. 128(2):203-07 [1996]), and with chronic diarrhea after liver transplantation in children. (D.R. Mack *et al.*, *Small bowel bacterial*

overgrowth as a cause of chronic diarrhea after liver transplantation in children, *Liver Transpl. Surg.* 4(2):166-69 [1998]).

Although diabetic enteropathy (F. Goldstein *et al.*, *Diabetic diarrhea and steatorrhea. Microbiologic and clinical observations*, *Ann. Intern. Med.* 1970;72:215-8 [1970]), idiopathic intestinal pseudo-obstruction (A.J. Pearson *et al.*, *Intestinal pseudo-obstruction with bacterial overgrowth in the small intestine*, *Am. J. Dig. Dis.* 14:200-05 [1969]) and scleroderma (I.J. Kahn *et al.*, *Malabsorption in intestinal scleroderma: Correction with antibiotics*, *N. Engl. J. Med.* 274: 1339-44 [1966]) are all known to produce motility disturbances leading to SIBO. Two previous reports have examined small bowel motility among anatomically and medically naive SIBO subjects. (G. Vantrappen *et al.*, *The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine*, *J. Clin. Invest.* 59: 1158-66 [1977]; P.O. Stotzer *et al.*, *Interdigestive and postprandial motility in small-intestinal bacterial overgrowth*, *Scand. J. Gastroenterol.* 31:875-80 [1996]). These authors suggest that the majority of subjects with SIBO in the absence of other predisposing conditions, lack the phase III of interdigestive motility during short term recordings.

Phase III of interdigestive motility is a period of phasic contractions propagating through the length of the small intestine, approximately once every 87.2 ± 5.4 minutes in the fasting state. (E.E. Soffer *et al.*, *Prolonged ambulatory duodeno-jejunal manometry in humans: Normal values and gender effect*, *Am. J. Gastrol.* 93:1318-23 [1998]). This fasting event is responsible for sweeping residue including small bowel contaminants, such as accumulated bacteria, into the colon in preparation for the next meal. (V.B. Nieuwenhuijs *et al.*, *The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats*, *Ann. Surg.* 228: 188-93 [1998]; E. Husebye, *Gastrointestinal motility disorders and bacterial overgrowth*, *J. Intern. Med.* 237:419-27 [1995]). The endogenous peptide, motilin, is involved in the mediation of this event. (G. Vantrappen *et al.*, *Motilin and the interdigestive migrating motor complex in man*, *Dig. Dis. Sci.* 24:497-500 [1979]). Other prokinetic agents, such as erythromycin, are believed to act on the motilin receptor and have been shown to rapidly induce an interdigestive motility event in dogs and humans. (M.F. Otterson and S.K. Sarna, *Gastrointestinal motor effect of erythromycin*, *Am. J. Physiol.* 259:G355-63; T. Tomomasa *et al.*, *Erythromycin induces migrating motor complex in human gastrointestinal tract*, *Dig. Dis. Sci.* 31:157-61 [1986]).

In general, the speed of transit through the small intestine is normally regulated by inhibitory mechanisms located in the proximal and distal small intestine known as the jejunal brake and the ileal brake. Inhibitory feedback is activated to slow transit when end products of digestion make contact with nutrient sensors of the small intestine. (E.g., Lin, H.C., U.S. Patent No. 5,977,175; Dobson, C. L. *et al.*, *The effect of oleic acid on the human ileal brake and its implications for small intestinal transit of tablet formulations*, Pharm. Res. 16(1):92-96 [1999]; Lin, H. C. *et al.*, *Intestinal transit is more potently inhibited by fat in the distal (Ileal brake) than in the proximal (jejunal brake) gut*, Dig. Dis. Sci. 42(1):19-25 [1997]; Lin, H.C. *et al.*, *Jejunal brake: inhibition of intestinal transit by fat in the proximal small intestine*, Dig. Dis. Sci., 41(2):326-29 [1996a]).

Specifically, jejunal and ileal brakes slow transit by the release of gut peptides such as peptide YY and by the activation of neural pathways such as those involving endogenous opioids. (Lin, H.C. *et al.*, *Fat-induced ileal brake in the dog depends on peptide YY*, Gastroenterol. 110(5):1491-95 [1996b]). Transit is then slowed by the stimulation of nonpropagative intestinal contractions which inhibit movement of the luminal content. The removal or impairment of these inhibitory mechanisms can lead to abnormally rapid transit. For example, in patients with a history of resection of the terminal ileum, intestinal transit can become uncontrolled and abnormally accelerated when the ileal brake is no longer intact. Time for processing of food can then be so reduced that few end products of digestion are available to trigger the jejunal brake as the remaining inhibitory mechanism.

Peptide YY and its analogs or agonists have been used to manipulate endocrine regulation of cell proliferation, nutrient transport, and intestinal water and electrolyte secretion. (E.g., Balasubramaniam, *Analogues of peptide yy and uses thereof*, U.S. Patent No. 5,604,203; WO9820885A1; EP692971A1; Croom *et al.*, *Method of enhancing nutrient uptake*, U.S. Patent No. 5,912,227; Litvak, D.A. *et al.*, *Characterization of two novel proabsorptive peptide YY analogs, BIM-43073D and BIM-43004C*, Dig. Dis. Sci. 44(3):643-48 [1999]). A role for peptide YY in the regulation of intestinal motility, secretion, and blood flow has also been suggested, as well as its use in a treatment of malabsorptive disorders (Liu, C.D. *et al.*, *Peptide YY: a potential proabsorptive hormone for the treatment of malabsorptive disorders*, Am. Surg. 62(3):232-36 [1996]; Liu, C.D. *et al.*, *Intraluminal peptide YY induces colonic absorption in vivo*, Dis. Colon Rectum 40(4):478-82 [1997]; Bilchik, A.J. *et al.*, *Peptide YY augments postprandial small intestinal absorption in the conscious dog*, Am. J. Surg. 167(6):570-74 [1994]).

Lin *et al.* immuno-neutralized peptide YY in vivo to block the ileal brake response and, thus, showed that it is mediated by peptide YY. (Lin, H. C. *et al.*, *Fat-induced ileal brake in the dog depends on peptide YY*, *Gastroenterology*, 110(5):1491-95 [1996b]). Serum levels of peptide YY increase during the ileal brake response to nutrient infusion into the distal ileum. (Spiller, R. C. *et al.*, *Further characterisation of the "ileal brake" reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY*, *Gut*, 29(8):1042-51 [1988]; Pironi, L. *et al.*, *Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY.*, *Gastroenterology*, 105(3):733-9 [1993]; Dreznik, Z. *et al.*, *Effect of ileal oleate on interdigestive intestinal motility of the dog*, *Dig. Dis. Sci.*, 39(7):1511-8 [1994]; Lin, C. D. *et al.*, *Interluminal peptide YY induces colonic absorption in vivo*, *Dis. Colon Rectum*, 40(4):478-82 [Apr 1997]). In contrast, in vitro studies have shown peptide YY infused into isolated canine ileum dose-dependently increased phasic circular muscle activity. (Fox-Threlkeld, J. A. *et al.*, *Peptide YY stimulates circular muscle contractions of the isolated perfused canine ileum by inhibiting nitric oxide release and enhancing acetylcholine release*, *Peptides*, 14(6):1171-78 [1993]).

Kreutter *et al.* taught the use of β_3 -adrenoceptor agonists and antagonists for the treatment of intestinal motility disorders, as well as depression, prostate disease and dyslipidemia (U.S. Patent No. 5,627,200).

Bagnol *et al.* reported the comparative immunovisualization of mu and kappa opioid receptors in the various cell layers of the rat gastrointestinal tract, including a comparatively large number of kappa opioid receptors in the myenteric plexus. (Bagnol, D. *et al.*, *Cellular localization and distribution of the cloned mu and kappa opioid receptors in rat gastrointestinal tract*, *Neuroscience*, 81(2):579-91[1997]). They suggested that opioid receptors can directly influence neuronal activity in the gastrointestinal tract.

Kreek *et al.* taught the use of opioid receptor antagonists, such as naloxone, naltrexone, and nalmefene, for the relief of gastrointestinal dysmotility. (Kreek *et al.*, *Method for controlling gastrointestinal dysmotility*, U.S. Patent No. 4,987,136). Riviere *et al.* taught the use of the opioid receptor antagonist fedotozine in the treatment of intestinal obstructions (Riviere, P.J.M. *et al.*, US. Patent No. 5,362,756). Opioid-related constipation, the most common chronic adverse effect of opioid pain medications in patients who require long-term opioid administration, such as patients with advanced cancer or participants in methadone maintenance, has been treated with orally administered methylnaltrexone and

naloxone. (Yuan, C.S. *et al.*, *Methylnaltrexone for reversal of constipation due to chronic methadone use: a randomized controlled trial*, JAMA 283(3):367-72 [2000]; Meissner, W. *et al.*, *Oral naloxone reverses opioid-associated constipation*, Pain 84(1):105-9 [2000]; Culpepper-Morgan, J.A., *et al.*, *Treatment of opioid-induced constipation with oral naloxone: a pilot study*, Clin. Pharmacol. Ther. 52(1):90-95 [1992]; Yuan, C.S. *et al.*, *The safety and efficacy of oral methylnaltrexone in preventing morphine-induced delay in oral-cecal transit time*, Clin. Pharmacol. Ther. 61(4):467-75 [1997]; Santos, F. A. *et al.*, *Quinine-induced inhibition of gastrointestinal transit in mice: possible involvement of endogenous opioids*, Eur. J. Pharmacol., 364(2-3):193-97 [1999]. Naloxone was also reported to abolish the ileal brake in rats (Brown, N. J. *et al.*, *The effect of an opiate receptor antagonist on the ileal brake mechanism in the rat*, Pharmacology, 47(4):230-36 [1993]).

Receptors for 5-hydroxytryptamine (5-HT) have been localized on various cells of the gastrointestinal tract. (Gershon, M. D., *Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel*, Aliment. Pharmacol. Ther., 13 Suppl 2:15-30 [1999]; Kirchgessner, A. L. *et al.*, *Identification of cells that express 5-hydroxytryptamine_{1A} receptors in the nervous systems of the bowel and pancreas*, J. Comp. Neurol., 15:364(3):439-455 [1996]). Brown *et al.* reported that subcutaneous administration of 5-HT₃ receptor antagonists, granisetron and ondansetron, in rats delayed intestinal transit of a baked bean meal but abolished the ileal brake induced by ileal infusion of lipid. They postulated the presence of 5-HT₃ receptors on afferent nerves that initiate reflexes that both accelerate and delay intestinal transit. (Brown, N.J. *et al.*, *Granisetron and ondansetron: effects on the ileal brake mechanism in the rat*, J. Pharm. Pharmacol. 45(6):521-24 [1993]). Kuemmerle *et al.* reported neuro-endocrine 5-HT-mediation of motilin-induced accelerated gastrointestinal motility. (Kuemmerle, J. F. *et al.*, *Serotonin neural receptors mediate motilin-induced motility in isolated, vascularly perfused canine jejunum*, J. Surg. Res., 45(4):357-62 [1988]).

Ninety-five percent of the human body's stores of 5-hydroxytryptamine (5-HT), also known as serotonin, are found in the gastrointestinal tract. (Gershon, M. D., *The Second Brain*, New York: Harper Collins [1998]). In the intestines, the vast majority of 5-HT is located in the enterochromaffin (EC) cells of the mucosa (Gershon [1998]). 5-HT is also released by myenteric 5-HT neurons in the myenteric plexus. (Gershon, M. D., *The enteric nervous system*, Annu Rev Neurosci 4: 227-272 [1981]; Gershon, M. D. *et al.*, *Serotonin: synthesis and release from the myenteric plexus of the mouse intestine*, Science 149: 197-199

[1965]; Holzer, P., and G. Skofitsch, *Release of endogenous 5-hydroxytryptamine from the myenteric plexus of the guinea-pig isolated small intestine*, Br J Pharmacol 81: 381-386 [1984]; Penttilä, A., *Histochemical reactions of the enterochromaffin cells and the 5-hydroxytryptamine content of the mammalian duodenum*, Acta Physiol Scand Suppl 281: 1-

77 [1966]). These intrinsic 5-HT neurons receive input from parasympathetic and sympathetic fibers (Gershon, M. D., and D. L. Sherman, *Noradrenergic innervation of serotonergic neurons in the myenteric plexus*, J Comp Neurol 259: 193-210 [1987]) and provide input to the motor neurons in their vicinity to suggest that they are interneurons. 5-HT₃ receptors are widely expressed by these myenteric 5-HT neurons as well as their neighboring neurons (Galligan, J. J., *Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurons*, Behav Brain Res 73: 199-201 [1996]; Zhou, X., and J. J. Galligan, *Synaptic activation and properties of 5-hydroxytryptamine(3) receptors in myenteric neurons of guinea pig intestine*, J Pharmacol Exp Ther 290: 803-10 [1999]). However, the physiologic function of these myenteric 5-HT neurons is not known. (E.G., Gershon, M. D. *Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel*, Aliment Pharmacol Ther 13 Suppl 2: 15-30, 1999; Grider, J. R. *et al.*, *5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT₄/5-HT_{1p} receptors on sensory CGRP neurons*, Am J Physiol 270: G778-G782 [1996]).

Regardless of the source of 5-HT (mucosal vs. neuronal or both), the signaling role of this molecule is facilitated by the availability of a 5-HT reuptake transporter called SERT that terminates the signal with its removal. (Wade, P. R. *et al.*, *Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract*, J Neurosci 16: 2352-64 [1996]). Since SERT is a part of the plasma membrane of serotonergic neurons (Blakely, R. D. *et al.*, *Cloning and expression of a functional serotonin transporter from rat brain*, Nature 354: 66-70 [1991]), these transporters are ideally positioned to remove neuronal 5-HT after signaling is completed. Serotonergic nerves are, however, absent from the intestinal mucosa. (Furness, J. B., and M. Costas, *The enteric nervous system*, New York: Churchill Livingstone [1987]). Instead, mucosal 5-HT from EC cells is removed by SERT expressed by neighboring epithelial cells. (Chen, J. X. *et al.*, *Guinea pig 5-HT transporter: cloning, expression, distribution, and function in intestinal sensory reception*, Am J Physiol 275: G433-G448 [1998]).

The action of SERT is blocked by drugs that inhibit the reuptake transporter. These serotonin-selective reuptake inhibitors (SSRI) are widely used as antidepressants. The most

commonly prescribed example is fluoxetine (Prozac). These agents significantly alter the peristaltic response. Wade *et al.* reported that fluoxetine initially accelerated the passage of a pellet through an isolated segment of guinea pig colon to suggest potentiation of the peristaltic effect of 5-HT when the removal of this molecule was inhibited (Wade *et al.* [1996]). However, as the dose of the SSRI was increased, the transit of the pellet became slower and slower. This observation with fluoxetine suggested to Gershon that 5-HT receptors became desensitized when an excess of 5-HT stayed around for a longer period of time and traversed further away from its mucosal source (Gershon [1998]). These are then the current concepts to explain the common gastrointestinal side effects of SSRIs including nausea (excess 5-HT acting on extrinsic sensory nerves) and diarrhea (excess 5-HT acting on intrinsic primary afferent neurons to initiate peristalsis; Gershon [1998]).

The current scientific foundation for understanding the role of serotonin in normal and abnormal motility of the small intestine has been based on the role of mucosal serotonin in two enteric functions. The first is as the neurotransmitter, via the activation of intrinsic primary afferent neurons (IPAN), for the peristaltic reflex, which mediates colonic evacuation, and for the mucosal secretory reflex. (E.g., Grider, J. R. *et al.*, *5-Hydroxytryptamine₄ receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine*, *Gastroenterology*, 115(2):370-80 [1998]; Jin, J. G. *et al.*, *Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT₄ and 5-HT₃ receptors*, *J. Pharmacol. Exp. Ther.*, 288(1):93-97 [1999]; Foxx-Orenstein, A. E. *et al.*, *5-HT₄ receptor agonists and delta-opioid receptor antagonists act synergistically to stimulate colonic propulsion*, *Am J. Physiol.*, 275(5 Pt. 1):G979-83 [1998]; Foxx-Orenstein, A. E., *Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine*, *Gastroenterology* 111(5):1281-90 [1996]; Wade, P. R. *et al.*, *Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract*, *J. Neurosci.*, 16(7):2352-64 [1996]; Grider, J., *Gastrin-releasing peptide (GRP) neuron are excitatory neurons in the descending phase of the peristaltic reflex*, *Gastroenterology* 116: A1000 [1999]; Cooke, H., M. Sidhu, and Y. Wang, *5-HT activates neural reflexes regulating secretion in the guinea pig colon*, *Neurogastroenterol Motil* 9: 181-6 [1997]; Cooke, H. J., and H. V. Carey, *Pharmacological analysis of 5-hydroxytryptamine actions on guinea-pig ileal mucosa*, *Eur J Pharmacol* 111: 329-37, [1985]; Frieling, T., J. Wood, and H. Cooke, *Submucosal reflexes: distension-evoked ion transport in the guinea pig distal colon*, *Am J Physiol* 263: G91-96 [1992]; Hardcastle, J., and P. Hardcastle, *Comparison of the intestinal*

secretory responses to 5-hydroxytryptamine in the rat jejunum and ileum in-vitro, J Pharm Pharmacol 49: 1126-31 [1997]; Kinsman, R. I., and N. W. Read, *Effect of naloxone on feedback regulation of small bowel transit by fat*, Gastroenterology 87: 335-337 [1984]).

The second enteric role for 5-HT is as the signal to the brain about lumenal conditions, linking mucosal stimuli with the brain via extrinsic primary sensory neurons. (Blackshaw, L. A., and D. Grundy, *Effects of 5-hydroxytryptamine on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret*, J Auton Nerv Syst 45: 41-50 [1993]). On the basis of this understanding, concepts have evolved to explain the irritable bowel syndrome as a condition of serotonin excess (leading to diarrhea from excessive peristalsis) (Gershon [1998]), even as the constipation typical of this syndrome remains puzzling. Similar explanations have also been used to explain the diarrhea reported by patients taking SSRI (e.g. Prozac).

The intestinal response to 5-HT has previously been described in terms of the peristaltic reflex in in vitro models: Bulbring and Crema first showed that lumenal 5-HT resulted in peristalsis. (Bulbring *et al.*, J. Physiol. 140:381-407 [1959]; Bulbring *et al.*, Brit. J. Pharm. 13:444-457 [1958]). Since the stimulation of peristalsis by 5-HT was unaffected by extrinsic denervation (Bulbring *et al.*, QJ Exp. Physiol. 43:26-37 [1958]), the peristaltic reflex was considered to be intrinsic to the enteric nervous system. Using a modified Trendelenburg model that compartmentalized the peristaltic reflex into the sensory limb, the ascending contraction limb (orad to stimulus) and the descending relaxation limb (aborad to stimulus), Grider, *et al.* reported that (1) mucosal stimulation but not muscle stretch released 5-HT to activate a primary sensory neuron to release calcitonin gene-related peptide (CGRP)(Grider *et al.*, Am. J. Physiol. 270:G778-G782 [1996]) via 5-HT₄ receptors in humans and rats (also 5-HT_{1p} in rats) and 5-HT₃ receptors in guinea pigs; (2) cholinergic interneurons are then stimulated by CGRP to initiate both ascending contraction via an excitatory motor neuron that depends on substances P and K and acetylcholine (Grider *et al.*, Am. J. Physiol. 257:G709-G714 [1989]) and descending relaxation (Grider, Am. J. Physiol. 266:G1139-G1145 [1994]; Grider *et al.* [1996], Jin *et al.*, J. Pharmacol. Exp. Ther. 288:93-97 [1999]) via an inhibitory motor neuron that depends on pituitary adenylate cyclase-activating peptide (PACAP), nitric oxide and vasoactive inhibitory peptide (VIP)(Grider *et al.*, Neuroscience 54:521-526 [1993]; Grider *et al.*, J. Auton. Nerv. Syst. 50:151-159 [1994]); and (3) peristalsis is controlled by [a] an opioid pathway that inhibits descending relaxation by suppressing the release of VIP; [b] a somatostatin pathway that inhibits this opioid pathway

(Grider, Am. J. Physiol. 275:G973-G978 [1998]); and [c] a GABA (Grider, Am. J. Physiol. 267:G696-G701 [1994]) and a gastrin releasing peptide (GRP) (Grider, Gastroenterol. 116:A1000 [1999]) pathway that stimulate VIP release. An opioid pathway that inhibits the excitatory motor neurons responsible for ascending contraction has also been described
5 (Gintzler *et al.*, Br. J. Pharmacol. 75:199-205 [1982]; Yau *et al.*, Am. J. Physiol. 250:G60-G63 [1986]). These observations are consistent with neuroanatomic and electrophysiological observations.

In addition, mucosal stroking has been found to induce 5-HT release by intestinal mucosal cells, which in turn activates a 5-HT₄ receptor on enteric sensory neurons, evoking
10 a neuronal reflex that stimulates chloride secretion (Kellum, J.M. *et al.*, Stroking human jejunal mucosa induces 5-HT release and Cl⁻ secretion via afferent neurons and 5-HT₄ receptors, Am. J. Physiol. 277(3 Pt 1):G515-20 [1999]).

Agonists of 5-HT₄/5, 5-HT₃ receptors, as well as opioid Δ receptor antagonists, were reported to facilitate peristaltic propulsive activity in the colon in response to
15 mechanical stroking, which causes the endogenous release of 5-HT and calcitonin gene-related protein (CGRP) in the stroked mucosal area. (Steadman, C.J. *et al.*, *Selective 5-hydroxytryptamine type 3 receptor antagonism with ondansetron as treatment for diarrhea-predominant irritable bowel syndrome: a pilot study*, Mayo Clin. Proc. 67(8):732-38 [1992]).

Colonic distension also results in CGRP secretion, which is associated with triggering the
20 peristaltic reflex. 5-HT₃ receptor antagonists have been used for the treatment of autism. (E.g., Oakley *et al.*, *5-HT₃ receptor antagonists for the treatment of autism*, U.S. Patent No. 5,225,407).

Improved methods of detecting or diagnosing SIBO and SIBO-caused conditions are also a desideratum. Typically, detection of SIBO is done by detecting hydrogen and/or
25 methane exhaled in the the breath. (E.g., P. Kerlin and L. Wong, *Breath hydrogen testing in bacterial overgrowth of the small intestine*, Gastroenterol. 95(4):982-88 [1988]; A. Strocchi *et al.*, *Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H₂ criteria*, Gastroenterol. 105(5):1404-1410 [1993]; D. de Boissieu *et al.*, [1996]; P.J. Lewindon *et al.*, *Bowel dysfunction in cystic fibrosis: importance of breath testing*, J.
30 Paediatr. Child Health 34(1):79-82 [1998]). Hydrogen is a metabolic product of the fermentation of carbohydrates and amino acids by bacteria normally found in the colon. While the hydrogen that is produced in the colonic lumen may be excreted via the lungs (exhaled breath) and the anus (flatus), these routes of excretion are responsible for the

elimination of only a fraction of the total amount of hydrogen (10%) that is produced in the gut (Levitt, M.D. *et al.*, *Hydrogen (H₂) catabolism in the colon of the rat*, J Lab clin Med 84:163-167 [1974]).

5 The major mechanism for the removal of hydrogen produced by bacterial fermentation is the utilization of this gas by colonic bacteria that competes to use hydrogen via one of three hydrogen disposal pathways that are mutually exclusive. These pathways depend on the metabolism of methanogenic bacteria (Levitt, M.D. *et al.*, *H₂ excretion after ingestion of complex carbohydrates*, Gastroenterology 92:383-389 [1987]), acetogenic bacteria (Lajoie, R. *et al.*, *Acetate production from hydrogen and [c13] carbon dioxide by*
10 *the microflora of human feces*, Appl Environ Microbiol 54:2723-2727 [1988]) and sulfate-reducing bacteria (Gibson, G.R. *et al.*, *Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut*, J Appl Bacteriol 65:103-111 [1988]). Methanogenic bacteria are more efficient than the other colonic bacteria in the elimination of luminal hydrogen. (Strocchi, A. *et al.*,
15 *Methanogens outcompete sulphate reducing bacteria for H₂ in the human colon*, Gut 35:1098-1101 [1994]). Acetogenic bacteria are uncommon, being found in the intestinal populations of <5% of humans.

In the colon, sulfate-reducing bacteria reduces sulfate to hydrogen sulfide. (MacFarlane, G.T. *et al.*, *Comparison of fermentation reactions in different regions of the*
20 *human colon*, J Appl Bacteriol 72:57-64 [1992]). Hydrogen sulfide is more damaging to tissues than anionic sulfide or sulfhydryl compounds. Intestinal bicarbonate facilitates the conversion of hydrogen sulfide produced by sulfate-reducing bacteria in the gut to anionic sulfide. (Hamilton WA: *Biocorrosion: The action of sulphate-reducing bacteria*, in *Biochemistry of Microbial Degradation*, C. Ratlidge (ed.) Dordrecht, Kluwer Academic
25 Publishers, pages 555-570 [1994]). Since sulfate-reducing bacteria are more common in patients with the diagnosis of ulcerative colitis (Pitcher, M.C.L. *et al.*, *Incidence and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis*, FEMS Microbiol Ecol 86:103-112 [1991]), sulfate-reducing bacteria have been considered for a possible role in the pathogenesis of ulcerative colitis. (Florin, R.H.J.
30 *et al.*, *A role for sulfate reducing bacteria in ulcerative colitis?*, Gastroenterology 98:A170 [1990]). This link has been postulated to be related to the injurious effect of hydrogen sulfide in impairing the use of short chain fatty acids as fuel by colonic epithelial cells. (Roediger, W.E.W. *et al.*, *Sulphide impairment of substrate oxidation in rat colonocytes: a biochemical*

basis for ulcerative colitis?, Clin Sci 85:623-627 [1993]; Roediger, W.E. *et al.*, *Reducing sulfur compounds of the colon impair colonocyte nutrition: implication of ulcerative colitis*, Gastroenterology 1993;104:802-809).

Currently, clinical detection of sulfur-containing gases is limited to the detection of halitosis or bad breath. (Rosenberg, M. *et al.*, *Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor*, J Dent Res. 1991 Nov;70(11):1436-40). After garlic ingestion, the presence of allyl methyl sulfide differentiates the intestine rather than the mouth as the source of the sulfur-containing volatile gas (Suarez, F. *et al.*, *Differentiation of mouth versus gut as site of origin of odoriferous breath gases after garlic ingestion*, Am J Physiol 276(2 pt 1):G425-30 [1999]).

The role of sulfate-reducing bacteria in small intestinal bacterial overgrowth has not been studied, and the presence of sulfate-reducing bacteria are not detected using the standard breath testing method which typically detects only the presence of hydrogen, methane and carbon dioxide.

There remains a need for an underlying causal factor, to which diagnostic testing and treatment can be directed, for SIBO and SIBO-caused conditions, such as irritable bowel syndrome; fibromyalgia; chronic pelvic pain syndrome; chronic fatigue syndrome; autism; depression; impaired mentation and/or memory; sugar craving; ADHD; MS, SLE and other autoimmune diseases; and Crohn's disease. This and other benefits of the present invention are described herein.

SUMMARY OF THE INVENTION

The present invention relates to the diagnosis or treatment of small intestinal bacterial overgrowth (SIBO) and SIBO-caused conditions. SIBO-caused conditions, as described herein, include irritable bowel syndrome (IBS), Crohn's disease (CD), fibromyalgia (FM), chronic pelvic pain syndrome (CPPS), chronic fatigue syndrome (CFS), depression, impaired mentation, impaired memory, halitosis, tinnitus, sugar craving, autism, attention deficit/hyperactivity disorder (ADHD), drug sensitivity, and autoimmune diseases, for example, multiple sclerosis (MS), systemic lupus erythematosus (SLE).

In particular, the present invention relates to a method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject. The method involves detecting in the subject by any suitable detection means, the presence or absence of SIBO in the subject. If SIBO is detected in the subject, the method further involves depriving

the bacterial population, which constitutes the overgrowth in the small intestine, of nutrient(s), sufficiently to inhibit the further growth of the bacteria in the small intestine.

With the growth of the bacteria constituting the SIBO condition thus inhibited, SIBO is at

least partially eradicated, as the subject's phase III interdigestive motility is better able to

5 clear the small intestine of the overgrowth and sweep the bacteria into the colon for eventual elimination from the body. In addition, the at least partial eradication of the SIBO condition

also decreases the occurrence or magnitude of bacteria-related toxicity, sepsis (in more severe or advanced SIBO), and/or the subject's own immune responses, which are continually

triggered by the presence of SIBO in non-immunocompromised subjects. The clinical

10 symptoms of the subject associated with SIBO or the SIBO-caused condition are, consequently, ameliorated by the at least partial eradication of SIBO.

In an alternative aspect of the present invention, the method involves inhibiting the

growth of the bacteria in the subject's small intestine, which bacteria constitute a SIBO condition that has been detected, by introducing into the lumen of the small intestine, a

15 pharmaceutically acceptable disinfectant or antibiotic composition in an amount sufficient to inhibit the growth of the bacteria, thereby at least partially eradicating SIBO in the human subject.

In still another alternative aspect of the present invention, the method of treating small intestinal bacterial overgrowth (SIBO), or a SIBO-caused condition, in a human subject

20 involves administering to the subject a pharmaceutically acceptable composition comprising a stabilizer of mast cell membranes in the luminal wall, in an amount sufficient to inhibit a mast cell-mediated immune response to SIBO in the human subject.

The present invention also relates to a method of screening for the abnormally likely presence of SIBO in a human subject. The method involves obtaining a serum sample from

25 the subject, and then quantitatively determining a concentration in the serum sample of serotonin, one or more unconjugated bile acid(s), and/or folate. An abnormally elevated serum concentration of one or more of these substances is indicative of a higher than normal probability that SIBO is present in the subject. Thus, if the method of screening for the

presence of SIBO is employed as part of a blood work-up, either as part of a routine physical

30 or by way of investigating a particular clinical complaint of the subject's, the practitioner can be made aware that SIBO is more than normally likely to be present. The practitioner can then elect to pursue a less convenient, but more diagnostically powerful, detection means for SIBO.

The present invention also relates to such a diagnostically powerful SIBO detection means. In particular, this inventive method of detecting small intestinal bacterial overgrowth in a human subject involves detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by the human subject, after the subject has
5 ingested a controlled quantity of a substrate. The exhaled gas mixture is at least partially produced by the metabolic activity of the intestinal microflora of the subject.

The present invention is also directed to a method of determining the relative severity of SIBO or a SIBO-caused condition in a human subject in whom SIBO has been detected.

The method involves detecting in the subject by suitable detection means, the presence or
10 absence of SIBO, and, if the presence of SIBO is detected in the subject, the method further involves detecting in the subject by suitable detection means a relative level of intestinal permeability, abnormally high intestinal permeability indicating a relatively severe SIBO or SIBO-caused condition in the subject.

The present invention also relates to a kit for the diagnosis of SIBO or a SIBO-caused
15 condition, comprising: at least one breath sampling container, a pre-measured amount of a substrate, and instructions for a user in detecting the presence or absence of SIBO by determining the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by the subject, after ingestion of a controlled quantity of the substrate. Thus, the kit is particularly useful in practicing the inventive method of detecting
20 small intestinal bacterial overgrowth in a human subject.

These and other advantages and features of the present invention will be described more fully in a detailed description of the preferred embodiments which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 shows visual analog scores reported by subjects with IBS and SIBO before and after antibiotic treatment.

Figure 2 shows visual analog scores from subjects with IBS and SIBO in a pilot study, before and after antibiotic treatment.

Figure 3 shows visual analog scores reported by subjects with fibromyalgia and SIBO
30 before and after antibiotic treatment.

Figure 4 shows the correlation between the degree of improvement in symptoms and residual breath hydrogen production after antibiotic treatment in subjects with fibromyalgia and SIBO.

Figure 5 shows visual analog scores reported by subjects with Crohn's disease and SIBO before and after antibiotic treatment.

Figure 6 shows the correlation between degree of improvement in symptoms and residual breath hydrogen production after antibiotic treatment in subjects with Crohn's disease.

Figure 7 shows that the severity of diarrheal symptoms is comparatively less in SIBO patients who excrete methane.

Figure 8 shows a typical effect of total enteral nutrition (TEN) regimen in the eradication of SIBO as detected by LBHT. In Figure 8A (pre-treatment), SIBO was initially detected. After 14 days of the TEN regimen, follow-up LBHT shows that SIBO had been at least partially eradicated (Figure 8B).

Figure 9 demonstrates that slowing of the rate of intestinal transit by fat depends on peptide YY (PYY), which is a physiological fat signal molecule.

Figure 10 demonstrates that slowing of the rate of intestinal transit by fat depends on a serotonergic pathway.

Figure 11 illustrates that the fat induced ileal brake depends on an ondansetron-sensitive, efferent serotonergic 5-HT₃-mediated pathway.

Figure 12 shows that ondansetron abolishes the fat-induced ileal brake in a dose-dependent fashion.

Figure 13 shows that ondansetron abolishes the fat-induced ileal brake when administered luminally but not intravenously.

Figure 14 illustrates that the slowing of intestinal transit by distal gut 5-HT depends on an ondansetron-sensitive 5-HT-mediated pathway in the proximal (efferent) and distal (afferent) gut.

Figure 15 shows that luminal 5-HT, delivered to the proximal gut, slows intestinal transit in a dose-dependent fashion.

Figure 16 illustrates that luminal 5-HT slows intestinal transit via activation of an intestino-intestinal reflex.

Figure 17 illustrates that slowing of intestinal transit by distal gut fat depends on an extrinsic adrenergic neural pathway.

Figure 18 illustrates that slowing of intestinal transit by PYY depends on an extrinsic adrenergic neural pathway.

Figure 19 illustrates that slowing of intestinal transit by 5-HT in the distal gut depends on a propranolol-sensitive extrinsic adrenergic neural pathway.

Figure 20 illustrates that intestinal transit is slowed by norepinephrine (NE) in a 5-HT-mediated neural pathway.

5 Figure 21 illustrates that the fat-induced jejunal brake depends on the slowing effect of a naloxone-sensitive, opioid neural pathway.

Figure 22 illustrates that the fat-induced ileal brake depends on the slowing effect of an efferent, naloxone-sensitive, opioid neural pathway.

10 Figure 23 shows that slowing of intestinal transit by distal gut 5-HT depends on a naloxone-sensitive, opioid neural pathway.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to a method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, including a juvenile or
15 adult, of any age or sex.

The upper gastrointestinal tract of a human subject includes the entire alimentary canal, except the cecum, colon, rectum, and anus. While some digestive processes, such as starch hydrolysis, begin in the mouth and esophagus, of particular importance as sites of digestion are the stomach and small intestine (or "small bowel"). The small intestine includes
20 the duodenum, jejunum, and the ileum. As the term is commonly used in the art, the proximal segment of the small bowel, or proximal gut, comprises approximately the first half of the small intestine from the pylorus to the mid-gut. The distal segment, or distal gut includes approximately the second half, from the mid-gut to the ileal-cecal valve.

As used herein, "digestion" encompasses the process of breaking down large
25 biological molecules into their smaller component molecules, for example, proteins into amino acids. "Predigested" means that the process of digestion has already begun or has occurred prior to arrival in the upper gastrointestinal tract.

As used herein, "absorption" encompasses the transport of a substance from the intestinal lumen through the barrier of the mucosal epithelial cells into the blood and/or
30 lymphatic systems.

Small intestinal bacterial overgrowth (SIBO), is an abnormal condition in which aerobic and anaerobic enteric bacteria from the colon proliferate in the small intestine, which is normally relatively free of bacterial contamination. SIBO is defined as greater than 10^6

CFU/mL small intestinal effluent. (R.M. Donaldson, Jr., *Normal bacterial populations of the intestine and their relation to intestinal function*, N. Engl. J. Med. 270:938-45 [1964]).

Typically, the symptoms of SIBO include abdominal pain, bloating, gas and alteration in bowel habits, such as constipation and diarrhea. SIBO-caused conditions is used herein interchangeably with the term "SIBO-related conditions," and regardless of ultimate causation, is a condition associated with the presence of SIBO in the subject. SIBO-caused conditions include other common symptoms, such as halitosis ("bad breath"), tinnitus (the experience of noise in the ears, such as ringing, buzzing, roaring, or clicking, which may not be associated with externally produced sounds), sugar craving, i.e., an intense desire for sweet foods or flavors, which can result in abnormally large consumption of sweet foods and beverages and frequently leads to health-threatening obesity. Drug sensitivity is another common SIBO-caused condition, in which the subject is hypersensitive to medications, such as non-steroidal anti-inflammatory medications, anti-insomniacs, antibiotics, or analgesics, and can suffer an unpredictable allergic-type reaction to medications at doses that normally do not adversely affect the vast majority of patients. It is a benefit provided by the present invention that it provides a useful solution in the present tense, for many patients, to the problem of drug sensitivity, without requiring complex pharmacogenetic research and customized drug development.

Other SIBO-caused conditions, as described herein, can include those falling in the diagnostic categories of irritable bowel syndrome, Crohn's disease, fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, depression, impaired mentation (including impairment of the ability to concentrate, calculate, compose, reason, and/or use foresight or deliberate judgment), impaired memory, autism, attention deficit/hyperactivity disorder, and/or autoimmune diseases, such as systemic lupus erythematosus (SLE) or multiple sclerosis (MS).

In accordance with the invention, the SIBO-caused condition can be, but need not be, previously diagnosed or suspected. The skilled medical practitioner is aware of suitable up-to-date diagnostic criteria by which a suspected diagnosis is reached. These diagnostic criteria are based on a presentation of symptom(s) by a human subject. For example, these criteria include, but are not limited to, the Rome criteria for IBS (W.G. Thompson, *Irritable bowel syndrome: pathogenesis and management*, Lancet 341:1569-72 [1993]) and the criteria for CFS established by the Centers for Disease Control and Prevention (CDC). (K. Fukuda *et al.*, *The chronic fatigue syndrome: a comprehensive approach to its definition and study*,

Ann. Intern. Med. 121:953-59 [1994]). The diagnostic criteria for fibromyalgia of the American College of Rheumatology will also be familiar (F. Wolfe *et al.*, *The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia: Report of the Multicenter Criteria Committee*, Arthritis Rheum. 33:160-72 [1990]), as will be the criteria for depression or ADHD provided for example, by the Diagnostic and Statistical Manual (DSM)-IV or its current version. (E.g., G. Tripp *et al.*, *DSM-IV and ICD-10: a comparison of the correlates of ADHD and hyperkinetic disorder*, J. Am. Acad. Child Adolesc. Psychiatry 38(2):156-64 [1999]). Symptoms of systemic lupus erythematosus include the 11 revised criteria of the American College of Rheumatology, such as a typical malar or discoid rash, photosensitivity, oral ulcers, arthritis, serositis, or disorders of blood, kidney or nervous system. (E.M Tan *et al.*, *The 1982 revised criteria for the classification of systemic lupus erythematosus [SLE]*, Arthritis Rheum. 25:1271-77 [1982]). Appropriate diagnostic criteria for multiple sclerosis are also familiar (e.g., L.A. Rolak, *The diagnosis of multiple sclerosis*, Neuronal Clin. 14(1):27-43 [1996]), as are symptoms of Crohn's disease useful in reaching a suspected diagnosis. (e.g., J.M. Bozdech and R.G. Farmer, *Diagnosis of Crohn's disease*, Hepatogastroenterol. 37(1):8-17 [1990]; M. Tanaka and R.H. Riddell, *The pathological diagnosis and differential diagnosis of Crohn's disease*, Hepatogastroenterol. 37(1):18-31 [1990]; A.B. Price and B.C. Morson, *Inflammatory bowel disease: the surgical pathology of Crohn's disease and ulcerative colitis*, Hum. Pathol. 6(1):7-29 [1975]). The practitioner is, of course not limited to these illustrative examples for diagnostic criteria, but should use criteria that are current in the art.

Detection of the presence of SIBO in the human subject also corroborates the suspected diagnosis of the SIBO-caused condition, held by a qualified medical practitioner who, prior to the detection of SIBO in the human subject, suspects from more limited clinical evidence that the human subject has, for example, irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, chronic pelvic pain syndrome, depression, autism, ADHD, an autoimmune disease, or *Crohn's disease*. By applying the inventive diagnostic method the suspected diagnosis is corroborated, i.e., confirmed, sustained, substantiated, supported, evidenced, strengthened, affirmed or made more firm.

The inventive method of treating SIBO, or a SIBO-caused condition, involves first detecting the presence or absence of SIBO in the subject by suitable detection means. Detecting the presence or absence of SIBO is accomplished by any suitable means or method known in the art. For example, one preferred method of detecting SIBO is breath hydrogen

testing. (E.g., P. Kerlin and L. Wong, *Breath hydrogen testing in bacterial overgrowth of the small intestine*, Gastroenterol. 95(4):982-88 [1988]; A. Strocchi *et al.*, *Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H₂ criteria*, Gastroenterol. 105(5):1404-1410 [1993]; D. de Boissieu *et al.*, [1996]; P.J. Lewindon *et al.*,
5 *Bowel dysfunction in cystic fibrosis: importance of breath testing*, J. Paediatr. Child Health 34(1):79-82 [1998]). Breath hydrogen or breath methane tests are based on the fact that many obligately or facultatively fermentative bacteria found in the gastrointestinal tract produce detectable quantities of hydrogen or methane gas as fermentation products from a substrate consumed by the host, under certain circumstances. Substrates include sugars such
10 as lactulose, xylose, lactose, sucrose, or glucose. The hydrogen or methane produced in the small intestine then enters the blood stream of the host and are gradually exhaled.

Typically, after an overnight fast, the patient swallows a controlled quantity of a sugar, such as lactulose, xylose, lactose, or glucose, and breath samples are taken at frequent time intervals, typically every 10 to 15 minutes for a two- to four-hour period. Samples are
15 analyzed by gas chromatography or by other suitable techniques, singly or in combination. Plots of breath hydrogen in patients with SIBO typically show a double peak, i.e., a smaller early hydrogen peak followed by a larger hydrogen peak, but a single hydrogen peak is also a useful indicator of SIBO, if peak breath hydrogen exceeds the normal range of hydrogen for a particular testing protocol. (See, G. Mastropaolo and W.D. Rees, *Evaluation of the*
20 *hydrogen breath test in man: definition and elimination of the early hydrogen peak*, Gut 28(6):721-25 [1987]).

A variable fraction of the population fails to exhale appreciable hydrogen gas during intestinal fermentation of lactulose; the intestinal microflora of these individuals instead produce more methane. (G. Corazza *et al.*, *Prevalence and consistency of low breath H₂*
25 *excretion following lactulose ingestion. Possible implications for the clinical use of the H₂ breath test*, Dig. Dis. Sci. 38(11):2010-16 [1993]; S. M. Riordan *et al.*, *The lactulose breath hydrogen test and small intestinal bacterial overgrowth*, Am. J. Gastroentrol. 91(9):1795-1803 [1996]). Consequently, in the event of an initial negative result for breath hydrogen, or as a precaution, methane and/or carbon dioxide contents in each breath sample are
30 optionally measured, as well as hydrogen, or a substrate other than lactulose is optionally used. Also, acting as a check, the presence of SIBO is demonstrated by a relative decrease in peak hydrogen exhalation values for an individual subject after antimicrobial treatment, in accordance with the present invention, compared to pretreatment values.

Another preferred method of detecting bacterial overgrowth is by gas chromatography with mass spectrometry and/or radiation detection to measure breath emissions of isotope-labeled carbon dioxide, methane, or hydrogen, after administering an isotope-labeled substrate that is metabolizable by gastrointestinal bacteria but poorly digestible by the human host, such as lactulose, xylose, mannitol, or urea. (E.g., G.R. Swart and J.W. van den Berg, *¹³C breath test in gastrointestinal practice*, Scand. J. Gastroenterol. [Suppl.] 225:13-18 [1998]; S.F. Dellert *et al.*, *The ¹³C-xylose breath test for the diagnosis of small bowel bacterial overgrowth in children*, J. Pediatr. Gastroenterol. Nutr. 25(2):153-58 [1997]; C.E. King and P.P. Toskes, *Breath tests in the diagnosis of small intestinal bacterial overgrowth*, Crit. Rev. Lab. Sci. 21(3):269-81 [1984]). A poorly digestible substrate is one for which there is a relative or absolute lack of capacity in a human for absorption thereof or for enzymatic degradation or catabolism thereof.

Suitable isotopic labels include ¹³C or ¹⁴C. For measuring methane or carbon dioxide, suitable isotopic labels can also include ²H and ³H or ¹⁷O and ¹⁸O, as long as the substrate is synthesized with the isotopic label placed in a metabolically suitable location in the structure of the substrate, i.e., a location where enzymatic biodegradation by intestinal microflora results in the isotopic label being sequestered in the gaseous product. If the isotopic label selected is a radioisotope, such as ¹⁴C, ³H, or ¹⁵O, breath samples can be analyzed by gas chromatography with suitable radiation detection means. (E.g., C.S. Chang *et al.*, *Increased accuracy of the carbon-14 D-xylose breath test in detecting small-intestinal bacterial overgrowth by correction with the gastric emptying rate*, Eur. J. Nucl. Med. 22(10):1118-22 [1995]; C.E. King and P.P. Toskes, *Comparison of the 1-gram [¹⁴C]xylose, 10-gram lactulose-H₂, and 80-gram glucose-H₂ breath tests in patients with small intestine bacterial overgrowth*, Gastroenterol. 91(6):1447-51 [1986]; A. Schneider *et al.*, *Value of the ¹⁴C-D-xylose breath test in patients with intestinal bacterial overgrowth*, Digestion 32(2):86-91 [1985]).

Another preferred method of detecting small intestinal bacterial overgrowth is direct intestinal sampling from the human subject. Direct sampling is done by intubation followed by scrape, biopsy, or aspiration of the contents of the intestinal lumen, including the lumen of the duodenum, jejunum, or ileum. The sampling is of any of the contents of the intestinal lumen including material of a cellular, fluid, fecal, or gaseous nature, or sampling is of the luminal wall itself. Analysis of the sample to detect bacterial overgrowth is by conventional

microbiological techniques including microscopy, culturing, and/or cell numeration techniques.

Another preferred method of detecting small intestinal bacterial overgrowth is by endoscopic visual inspection of the wall of the duodenum, jejunum, and/or ileum.

5 The preceding are merely illustrative and non-exhaustive examples of methods for detecting small intestinal bacterial overgrowth.

Another suitable, and most preferred, means for detecting the presence or absence of SIBO is the present inventive method of detecting small intestinal bacterial overgrowth in a human subject, which involves detecting the relative amounts of methane, hydrogen, and
10 at least one sulfur-containing gas in a gas mixture exhaled by said human subject, after the subject has ingested a controlled quantity of a substrate. The inventive method of detecting small intestinal bacterial overgrowth is more likely than conventional breath tests described above to detect the presence of SIBO, because in some subjects a pattern exists that is termed "non-hydrogen, non-methane excretion" (see, e.g., Example 9c hereinbelow). This pattern
15 is the result of the subject having a bacterial population constituting the SIBO condition, in which a sulfate-reducing metabolic pathway predominates as the primary means for the disposition of dihydrogen. In that condition, the removal of the hydrogen can be so complete that there is little residual hydrogen or methane gas to be detected in the exhaled breath, compared to the amount of sulfur-containing gas, such as hydrogen sulfide or a volatile
20 sulfhydryl compound detectable by the inventive method of detecting small intestinal bacterial overgrowth.

In accordance with the inventive method of detecting small intestinal bacterial overgrowth, the substrate is preferably a sugar, as described hereinabove, and more preferably a poorly digestible sugar or an isotope-labeled sugar. The at least one sulfur-
25 containing gas is methanethiol, dimethylsulfide, dimethyl disulfide, an allyl methyl sulfide, an allyl methyl sulfide, an allyl methyl disulfide, an allyl disulfide, an allyl mercaptan, or a methylmercaptan.

Most preferably, the sulfur-containing gas is hydrogen sulfide or a sulfhydryl compound.

The detection or determination of the relative amounts of methane, hydrogen, and
30 at least one sulfur-containing gas in the exhaled gas mixture is accomplished by means or systems known in the art, preferably by means of gas chromatography (e.g., Brunette, D.M. *et al.*, *The effects of dentifrice systems on oral malodor*, J Clin Dent. 9:76-82 [1998]; Tangerman, A. *et al.*, *A new sensitive assay for measuring volatile sulphur compounds in*

human breath by Tenax trapping and gas chromatography and its application in liver cirrhosis, Clin Chim Acta 1983;May 9;130(1):103-110 [1983]) and/or a radiation detection system, if appropriate. Most preferably, mass spectrometry is employed to detect the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in the exhaled gas mixture. (E.g., Spanel P, Smith D., *Quantification of hydrogen sulphide in humid air by selected ion flow tube mass spectrometry*, Rapid Commun Mass Spectrom 14(13):1136-1140 [2000]). Combined gas chromatography and mass spectrometry (GC/MS) is also useful. (E.g., Chinivasagam, H.N. et al., *Volatile components associated with bacterial spoilage of tropical prawns*, Int J Food Microbiol 1998 June 30;42(1-2):45-55). Most preferably, but not necessarily, the detection system employed requires only a single sample of exhaled gas mixture for the detection of methane, hydrogen, and at least one sulfur-containing gas. Detection methods that separately detect methane, hydrogen, and/or at least one sulfur containing gas are also useful.

Thus, thin-layer chromatography or high pressure liquid chromatography can be useful for detection of volatile sulfur-containing compounds. (E.g., Tsiagbe, V.K. et al., *Identification of volatile sulfur derivatives released from feathers of chicks fed diets with various levels of sulfur-containing amino acids*, J Nutr 1987 117(11):18859-65[1987]).

Direct-reading monitors for sulfides based on the use of an electrochemical voltametric sensor or polarographic cell can also be employed. Typically, gas is drawn into a sensor equipped with an electrocatalytic sensing electrode. An electrical current is generated by an electrochemical reaction proportional to the concentration of the gas. The quantity of the gas is typically determined by comparing to a known standard.

In some embodiments of the inventive method of detecting SIBO in a human subject, before detection, volatile sulfur-containing gases are trapped in Tenax absorbent (e.g., Tangerman, A. et al., Clin Chim Acta May 9;130(1):103-110 [1983]; Heida, H. et al., *Occupational exposure and indoor air quality monitoring in a composting facility*, Am Ind Hyg Assoc J 56(1):39-43[1995]) or other solvent/absorbent system such as dinitrophenyl thioethers (Tsiagbe, V.K. et al. [1987]).

It generally takes about 2 to 3 hours of the subjects's time and a pre-test fast to accomplish breath testing for SIBO; thus, a quicker and more convenient screening method to determine those subjects most likely to have SIBO is desirable. Such a screening test allows the clinician to make a more informed decision as to which patients would likely

benefit from more definitive SIBO testing, as described above. This pre-screening reduces unnecessary inconvenience and expense for subjects who are unlikely to have SIBO.

Hence, the present invention provides a method of screening for the abnormally likely presence of SIBO in a human subject. By abnormally likely is meant a likelihood of SIBO greater than expected in the general population. The inventive screening method involves obtaining a serum sample from the subject, which conventionally involves a blood draw, followed by separation of the serum from the whole blood. Conventional immunochemical techniques, such as ELISA, employing commercially available reagents, are used to quantitatively determine a concentration in the serum sample of serotonin (5-HT), one or more unconjugated bile acids (e.g., total bile acids or individual bile acids, e.g., deoxycholic acid), and/or folate, an abnormally elevated serum concentration of one or more of these being indicative of a higher than normal probability that SIBO is present in the subject. Such quantitative immunochemical determinations of serum values are also made commercially (e.g., Quest Diagnostics-Nichols Institute, 33608 Ortega Highway, San Juan Capistrano, CA 92690).

For example, a normal range for serum 5-HT is up to about 0.5 nanograms per milliliter. The normal range for total bile acids in serum is about 4.0 to about 19.0 micromole per liter, and for deoxycholic acid the normal range is about 0.7 to about 7.7 micromoles per liter. Normal ranges for other unconjugated bile acids are also known. The normal range for serum folate is about 2.6 to about 20.0 nanograms per milliliter. In accordance with the inventive method of screening, subjects with at least one serum value beyond the normal range are thus more than normally likely to have SIBO present and are candidates for further diagnostic SIBO detection procedures.

The present invention also relates to a method of determining the relative severity of SIBO or a SIBO-caused condition in a human subject in whom SIBO has been detected by a suitable detection means, as described herein above. If the presence of SIBO is detected in the subject, then suitable detection means are employed to detect in the subject a relative level of intestinal permeability, compared to normal. Abnormally high intestinal permeability indicates a relatively severe SIBO or SIBO-caused condition in the subject, which alerts the clinician that a more aggressive SIBO treatment regimen is desirable.

Techniques for detecting intestinal permeability and normal intestinal permeability ranges are known. (E.g., Haase, A.M. *et al.*, *Dual sugar permeability testing in diarrheal disease*, J. Pediatr. 136(2):232-37 [2000]; Spiller, R.C. *et al.*, *Increased rectal mucosal*

endocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post dysenteric irritable bowel syndrome, Gut 47(6):804-11 [2000]; Smecuol, E. *et al.*, *Sugar tests detect celiac disease among first-degree relatives*, Am. J. Gastroenterol. 94(12):3547-52 [1999]; Cox, M.A. *et al.*, *Measurement of small intestinal permeability markers, lactulose and mannitol in serum: results in celiac disease*, Dig. Dis. Sci. 44(2):402-06 [1999]; Cox, M.A. *et al.*, *Analytical method for the quantitation of mannitol and disaccharides in serum: a potentially useful technique in measuring small intestinal permeability in vivo*, Clin. Chim. Acta 263(2):197-205 [1997]; Fleming, S.C. *et al.*, *Measurement of sugar probes in serum: an alternative to urine measurement in intestinal permeability testing*, Clin. Chem. 42(3):445-48 [1996]).

Briefly, intestinal permeability is typically accomplished by measuring the relative serum or urine levels of two sugars, after ingestion of controlled amounts by the subject. One of the sugars, for example mannitol, is chosen because it is more typically more easily absorbed through the intestinal mucosa than the other sugar, for example, lactulose. Then about two hours after ingestion, a serum or urine sample is taken, and the ratio of the two sugars is determined. The closer the ratio of the two sugars in the sample approaches the ratio originally ingested, the more permeable is the subject's intestine.

After the presence of SIBO has been detected in the subject, in accordance with the inventive method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, the proliferating bacterial population constituting the SIBO is deprived of nutrient(s) sufficiently to inhibit the growth of the bacteria in the small intestine, which results in at least partially eradicating SIBO in the human subject.

Depriving the bacterial population of nutrient(s) is accomplished by any of a number of means.

For example, in some embodiments of the method of treating SIBO or a SIBO-caused condition, the subject consumes for a sustained period, a diet consisting essentially of nutrients that upon arrival in the upper gastrointestinal tract of the subject, are at least partially predigested. The sustained period being sufficient to at least partially eradicate SIBO in the human subject is at least about three days, preferably about 7 to about 18 days, and more preferably about 10 to about 14 days.

In some embodiments of the method, the at least partially predigested nutrient(s) are contained in a commestible total enteral nutrition (TEN) formulation, which is also called an "elemental diet." Such formulations are commercially available, for example, Vivonex®

T.E.N. (Sandoz Nutrition, Minneapolis, MN) and its variants, or the like. (See, e.g., Example 11 hereinbelow). A useful total enteral nutrition formulation satisfies all the subject's nutritional requirements, containing free amino acids, carbohydrates, lipids, and all essential vitamins and minerals, but in a form that is readily absorbable in the upper gastrointestinal tract, thus depriving or "starving" the bacterial population constituting the SIBO of nutrients of at least some of the nutrients they previously used for proliferating. Thus, bacterial growth in the small intestine is inhibited.

In another embodiment of the inventive method, a pancreatic enzyme supplement is administered to the subject before or substantially simultaneously with a meal, such that nutrients contained in the meal are at least partially predigested upon arrival in the upper gastrointestinal tract of the subject by the activity of the pancreatic enzyme supplement. Useful pancreatic enzyme supplements are commercially available, commonly called "Pancreatin"; such supplements contain amylase, lipase, and/or protease. Representative methods of administering the pancreatic enzyme supplement include giving, providing, feeding or force-feeding, dispensing, inserting, injecting, infusing, prescribing, furnishing, treating with, taking, swallowing, ingesting, eating or applying.

In a preferred embodiment, depending on the formulation, the pancreatic enzyme supplement is administered up to a period of 24 hours prior to ingestion of the food or nutrient comprising the meal, but most preferably between about 60 to 0 minutes before ingestion, which is substantially simultaneously with the meal. The period of time prior to ingestion is determined on the precise formulation of the composition. For example, a controlled release formulation can be administered longer before the meal. Other quick release formulations can be taken substantially simultaneously with the meal.

In other embodiments of the method of treating small intestinal bacterial overgrowth or a SIBO-caused condition, depriving the bacterial population of nutrient(s) involves enhancing the digestion and/or absorption of the nutrient(s) in the upper gastrointestinal tract of the human subject by slowing transit of the nutrient(s) across the upper gastrointestinal tract of the human subject, thereby at least partially depriving the bacterial population of the nutrient(s). These embodiments of the inventive take advantage of a novel understanding of the peripheral neural connections that exist between the enteric nervous system of the upper gastrointestinal tract, including an intrinsic serotonergic neural pathway, and the vertebral ganglia, and thence to the central nervous system. The present invention provides a means to enhance region-to region (e.g., intestino-intestinal reflex) communications by way

of replicating 5-HT as a signal (or releasing 5-HT at a distance as a surrogate signal). Thus, the present invention provides a way to increase 5-HT in locations in the central nervous by transmitting a neural signal from the gut, or to transmit a 5-HT-mediated neural signal originating in one location in the gut via an intrinsic cholinergic afferent neural pathway to a second distant location in the gut where a serotonergic signal of the same or greater intensity is replicated.

The present technology, therefore, allows neurally mediated modulation of the rate of upper gastrointestinal transit in the human subject. The present invention allows the artificially directed transmission and/or amplification of nervous signals from one location in the enteric nervous system to another via a prevertebral ganglion, bypassing the central nervous system. The invention takes advantage of an intrinsic serotonergic neural pathway involving an intrinsic cholinergic afferent neural pathway that projects from peptide YY-sensitive primary sensory neurons in the intestinal wall to the prevertebral celiac ganglion. The prevertebral celiac ganglion is in turn linked by multiple prevertebral ganglionic pathways to the central nervous system, to the superior mesenteric ganglion, to the inferior mesenteric ganglion, and also back to the enteric nervous system via an adrenergic efferent neural pathway that projects from the prevertebral celiac ganglion to one or more enterochromaffin cells in the intestinal mucosa and to serotonergic interneurons that are, in turn, linked in the myenteric plexus or submucous plexus to opioid interneurons. The opioid interneurons are in turn linked to excitatory and inhibitory motoneurons. The opioid interneurons are also linked by an intestino-fugal opioid pathway that projects to the prevertebral celiac ganglion, with one or more neural connections therefrom to the central nervous system, including the spinal cord, brain, hypothalamus, and pituitary, and projecting back from the central nervous system to the enteric nervous system.

In particular, the present invention employs a method of manipulating the rate of upper gastrointestinal transit of food or nutrient substance(s). The method involves administering by an oral or enteral delivery route a pharmaceutically acceptable composition comprising an active agent to the upper gastrointestinal tract. To slow the rate of upper gastrointestinal transit, the active agent is an active lipid; a serotonin, serotonin agonist, or serotonin re-uptake inhibitor; peptide YY or a peptide YY functional analog; calcitonin gene-related peptide (CGRP) or a CGRP functional analog; an adrenergic agonist; an opioid agonist; or a combination of any of any of these, which is delivered in an amount and under conditions such that the cholinergic intestino-fugal pathway, at least one prevertebral

ganglionic pathway, the adrenergic efferent neural pathway, the serotonergic interneuron and/or the opioid interneuron are activated thereby. This results in the rate of upper gastrointestinal transit in the subject being slowed, which is the basis for prolonging the residence time of orally or enterally administered food or nutrient substances, thus promoting or enhancing their dissolution and/or absorption in the upper gastrointestinal tract.

The inventive pharmaceutically acceptable compositions limit the presentation of a food or nutrient substance to the proximal region of the small intestine for absorption.

Depending on the desired results, useful active agents include, active lipids; serotonin, serotonin agonists, or serotonin re-uptakeinhibitors; peptide YY or peptide YY functional analogs; CGRP or CGRP functional analogs; adrenergic agonists; opioid agonists; or a combination of any of any of these; antagonists of serotonin receptors, peptide YY receptors, adrenoceptors, opioid receptors, CGRP receptors, or a combination of any of these. Also useful are antagonists of serotonin receptors, peptide YY receptors, CGRP receptors; adrenoceptors and/or opioid receptors.

Serotonin, or 5-hydroxytryptamine (5-HT) is preferably used at a dose of about 0.03 to about 0.1 mg/kg of body mass. 5-HT₃ and 5-HT₄ serotonin receptor agonists are known and include HTF-919 and R-093877 (Foxy-Orenstein, A.E. et al., Am. J. Physiol. 275(5 Pt 1):G979-83 [1998]); prucalopride; 2-[1-(4-Piperonyl)piperazinyl]benzothiazole; 1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone; and 1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-2-methylsulphonylamino)ethyl-4-piperidinyl]-1-propanone. Serotonin re-uptake inhibitors include Prozac or Zoloft.

Useful serotonin receptor antagonists include known antagonists of 5-HT₃, 5-HT_{1P}, 5-HT_{1A}, 5-HT₂, and/or 5-HT₄ receptors. Examples include ondansetron or granisetron, 5HT₃ receptor antagonists (preferred dose range of about 0.04 to 5 mg/kg), deramciclane (Varga, G. et al., *Effect of deramciclane, a new 5-HT receptor antagonist, on cholecystokinin-induced changes in rat gastrointestinal function*, Eur. J. Pharmacol. 367(2-3):315-23 [1999]), or alosetron. 5-HT₄ receptor antagonists are preferably used at a dose of about 0.05 to 500 picomoles/kg. 5-HT₄ receptor antagonists include 1-PiperidinylethylH-indole-3-carboxylate (SB203186); 1-[4-Amino-5-chloro-2-(3,5-dimethoxyphenyl)methoxy]-3-[1-[2methylsulphonylamino]ethyl]piperidin-4-yl]propan-1-one (RS 39604); 3-(Piperidin-1-yl)propyl 4-amino-5-chloro-2-methoxybenzoate.

Peptide YY (PYY) and its functional analogs are preferably delivered at a dose of about 0.5 to about 500 picomoles/kg. PYY functional analogs include PYY (22-36), BIM-

43004 (Liu, CD. et al., J. Surg. Res. 59(1):80-84 [1995]), BIM-43073D, BIM-43004C (Litvak, D.A. et al., Dig. Dis. Sci. 44(3):643-48 [1999]). Other examples are also known in the art (e.g., Balasubramaniam, U.S. Patent No. 5,604,203).

PYY receptor antagonists preferably include antagonists of Y4/PP1, Y5 or
5 Y5/PP2/Y2, and most preferably Y1 or Y2. (E.g., Croom *et al.*, U.S. Patent No. 5,912,227) Other examples include BIBP3226, CGP71683A (King, P.J. et al., J. Neurochem. 73(2):641-46 [1999]).

CGRP receptor antagonists include human CGRP(8-37) (e.g., Foxx-Orenstein *et al.*, Gastroenterol. 111(5):1281-90 [1996]).

10 Useful adrenergic agonists include norepinephrine.

Adrenergic or adrenoceptor antagonists include β -adrenoceptor antagonists, including propranolol and atenolol. They are preferably used at a dose of 0.05-2 mg/kg.

Opioid agonists include delta-acting opioid agonists (preferred dose range is 0.05-50 mg/kg, most preferred is 0.05-25 mg/kg); kappa-acting opioid agonists (preferred dose
15 range is 0.005-100 microgram/kg); mu-acting opioid agonists (preferred dose range is 0.05-25 microgram/kg); and epsilon-acting agonists. Examples of useful opioid agonists include deltorphins (e.g., deltorphin II and analogues), enkephalins (e.g., [d-Ala(2), Gly-ol(5)]-enkephalin [DAMGO]; [D-Pen(2,5)]-enkephalin [DPDPE]), dinorphins, trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide methane sulfonate (U-50,
20 488H), morphine, codeine, endorphin, or β -endorphin.

Opioid receptor antagonists include mu-acting opioid antagonists (preferably used at a dose range of 0.05-5 microgram/kg); kappa opioid receptor antagonists (preferably used at a dose of 0.05-30 mg/kg); delta opioid receptor antagonists (preferably used at a dose of 0.05-200 microgram/kg); and epsilon opioid receptor antagonists. Examples of useful opioid
25 receptor antagonists include naloxone, naltrexone, methylnaltrexone, nalmefene, H2186, H3116, or fedotozine, i.e., (+)-1-1[3,4,5-trimethoxy]benzyloxymethyl-1-phenyl-N,N-dimethylpropylamine. Other useful opioid receptor antagonists are known (e.g., Kreek et al., U.S. Patent No. 4,987,136).

The active agents listed above are not exhaustive but rather illustrative examples, and
30 one skilled in the art is aware of other useful examples.

As used herein, "active lipid" encompasses a digested or substantially digested molecule having a structure and function substantially similar to a hydrolyzed end-product of fat digestion. Examples of hydrolyzed end products are molecules such as diglyceride, monoglyceride, glycerol, and most preferably free fatty acids or salts thereof.

5 In a preferred embodiment, the active agent is an active lipid comprising a saturated or unsaturated fatty acid. Fatty acids contemplated by the invention include fatty acids having between 4 and 24 carbon atoms (C4-C24).

Examples of fatty acids contemplated for use in the practice of the present invention include caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, oleic acid, palmitic
10 acid, stearic acid, palmitoleic acid, linoleic acid, linolenic acid, *trans*-hexadecanoic acid, elaidic acid, columbinic acid, arachidic acid, behenic acid, eicosenoic acid, erucic acid, bressidic acid, cetoleic acid, nervonic acid, Mead acid, arachidonic acid, timnodonic acid, clupanodonic acid, docosahexaenoic acid, and the like. In a preferred embodiment, the active lipid comprises oleic acid.

15 Also preferred are active lipids in the form of pharmaceutically acceptable salts of hydrolyzed fats, including salts of fatty acids. Sodium or potassium salts are preferred, but salts formed with other pharmaceutically acceptable cations are also useful. Useful examples include sodium- or potassium salts of caprolate, caprylate, caprate, laurate, myristate, oleate, palmitate, stearate, palmitolate, linolate, linolenate, *trans*-hexadecanoate, elaidate,
20 columbate, arachidate, behenate, eicosenoate, erucate, bressidate, cetoleate, nervonate, arachidonate, timnodonate, clupanodonate, docosahexaenoate, and the like. In a preferred embodiment, the active lipid comprises an oleate salt.

The active agents suitable for use with this invention are employed in well dispersed form in a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable
25 carrier" encompasses any of the standard pharmaceutical carriers known to those of skill in the art. For example, one useful carrier is a commercially available emulsion, Ensure, but active lipids, such as oleate or oleic acid are also dispersible in gravies, dressings, sauces or other comestible carriers. Dispersion can be accomplished in various ways. The first is that of a solution.

30 Lipids can be held in solution if the solution has the properties of bile (i.e., solution of mixed micelles with bile salt added), or the solution has the properties of a detergent (e.g., pH 9.6 carbonate buffer) or a solvent (e.g., solution of Tween). The second is an emulsion which is a 2-phase system in which one liquid is dispersed in the form of small globules

throughout another liquid that is immiscible with the first liquid (Swinyard and Lowenthal, "Pharmaceutical Necessities" *REMINGTON'S PHARMACEUTICAL SCIENCES*, 17th ed., AR Gennaro (Ed), Philadelphia College of Pharmacy and Science, 1985 p.1296). The third is a suspension with dispersed solids (e.g., microcrystalline suspension). Additionally, any
5 emulsifying and suspending agent that is acceptable for human consumption can be used as a vehicle for dispersion of the composition. For example, gum acacia, agar, sodium alginate, bentonite, carbomer, carboxymethylcellulose, carrageenan, powdered cellulose, cholesterol, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, octoxynol 9, oleyl alcohol, polyvinyl alcohol, povidone, propylene glycol
10 monostearate, sodium lauryl sulfate, sorbitan esters, stearyl alcohol, tragacanth, xantham gum, chondrus, glycerin, trolamine, coconut oil, propylene glycol, thyl alcohol malt, and malt extract.

Any of these formulations, whether it is a solution, emulsion or suspension containing the active agent, can be incorporated into capsules, or a microsphere or particle
15 (coated or not) contained in a capsule.

The pharmaceutically acceptable compositions containing the active agent, in accordance with the invention, is in a form suitable for oral or enteral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups, elixirs or enteral formulas. Compositions intended
20 for oral use are prepared according to any method known to the art for the manufacture of pharmaceutical compositions. Compositions can also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release. Other techniques for controlled release compositions, such as those described in the U.S. Pat. Nos. 4,193,985; and 4,690,822; 4,572,833 can be used in the
25 formulation of the inventive pharmaceutically acceptable compositions.

An effective amount of active lipid is any amount that is effective to slow gastrointestinal transit and control presentation of a food or nutrient substance to a desired region of the small intestine. For example, an effective amount of active lipid, as contemplated by the instant invention, is any amount of active lipid that can trigger any or
30 all of the following reflexes: intestino-lower esophageal sphincter (relaxation of LES); intestino-gastric feedback (inhibition of gastric emptying); intestino-intestinal feedback (ileo-jejunal feedback/ileal brake, jejuno-jejunal feedback/jejunal brake, intestino-CNS feedback (for example, intensifying intestinal signalling of satiety)); intestino-pancreatic feedback

(control of exocrine enzyme output); intestino-biliary feedback (control of bile flow); intestino-mesenteric blood flow feedback (for the control of mucosal hyperemia); intestino-colonic feedback (so called gastro-colonic reflex whereby the colon contracts in response to nutrients in the proximal small intestine).

5 Methods of administering are well known to those of skill in the art and include most preferably oral administration and/or enteral administration. Representative methods of administering include giving, providing, feeding or force-feeding, dispensing, inserting, injecting, infusing, perfusing, prescribing, furnishing, treating with, taking, swallowing, eating or applying. Preferably the pharmaceutically acceptable composition comprising the
10 active agent is administered in the setting of a meal, i.e., along with or substantially simultaneously with the meal, most preferably an hour or less before the meal. It is also useful to administer the active agent in the fasted state, particularly if the pharmaceutical composition containing the active agent is formulated for long acting or extended release. In some embodiments, such as the inventive method for manipulating post-prandial blood
15 flow, the pharmaceutical composition is also usefully administered up to an hour after a meal, and most preferably within one hour before or after the meal.

In order to stretch biologic activity so that one has a convenient, daily dosage regimen, the present invention contemplates that the inventive compositions can be administered prior to ingestion of the food, nutrient and/or drug.

20 In a preferred embodiment, the inventive compositions (depending on the formulation) are administered up to a period of 24 hours prior to ingestion of the food, nutrient and/or drug, but most preferably between about 60 to 5 minutes before ingestion. The period of time prior to ingestion is determined on the precise formulation of the composition. For example, if the formulation incorporates a controlled release system, the
25 duration of release and activation of the active lipid will determine the time for administration of the composition. Sustained release formulation of the composition is useful to ensure that the feedback effect is sustained.

In a preferred embodiment, the pharmaceutically acceptable composition of the invention contains an active lipid and is administered in a load-dependent manner which
30 ensures that the dispersion of active lipid is presented to the entire length of the small intestine. Administration is in one or more doses such that the desired effect is produced. In some preferred embodiments, the load of active lipid per dose is from about 0.5 grams to about 2.0 grams, but can range up to about 25 grams per dose as needed. Generally, patients

respond well to the most preferred amount of active lipid, which is in the range of about 1.6 to 3.2 grams. For patients who fail to respond to this dose range, a dose between 6 and 8 grams is typically effective.

Sequential dosing is especially useful for patients with short bowel syndrome or others with abnormally rapid intestinal transit times. In these patients, the first preprandial administration of the active lipid occurs in a condition of uncontrolled intestinal transit that can fail to permit optimal effectiveness of the active lipid. A second (or more) preprandial administration(s) timed about fifteen minutes after the first or previous administration and about fifteen minutes before the meal enhances the patient's control of intestinal luminal contents and the effectiveness of the active lipid in accordance with the inventive methods.

Normalization of nutrient absorption and bowel control throughout the day, including during the patient's extended sleeping hours, is best achieved by a dietary regimen of three major meals with about five snacks interspersed between them, including importantly, a pre-bedtime snack; administration of a dose of the inventive composition should occur before each meal or snack as described above.

Treatment with the inventive compositions in accordance with the inventive methods can be of singular occurrence or can be continued indefinitely as needed. For example, patients deprived of food for an extended period (e.g., due to a surgical intervention or prolonged starvation), upon the reintroduction of ingestible food, can benefit from administration of the inventive compositions before meals on a temporary basis to facilitate a nutrient adaptive response to normal feeding. On the other hand some patients, for example those with surgically altered intestinal tracts (e.g., ileal resection), can benefit from continued pre-prandial treatment in accordance with the inventive methods for an indefinite period. However, clinical experience with such patients for over six years has demonstrated that after prolonged treatment there is at least a potential for an adaptive sensory feedback response that can allow them to discontinue treatment for a number of days without a recurrence of postprandial diarrhea or intestinal dumping.

The use of pharmaceutically acceptable compositions of the present invention in enteral feeding contemplates adding the composition directly to the feeding formula. The composition can either be compounded as needed into the enteral formula when the rate of formula delivery is known (i.e., add just enough composition to deliver the load of active lipids). Alternatively, the composition of the invention can be compounded at the factory so that the enteral formulas are produced having different concentrations of the composition and

can be used according to the rate of formula delivery (i.e., higher concentration of composition for lower rate of delivery).

If the inventive composition were to be added to an enteral formula and the formula is continuously delivered into the small intestine, the composition that is initially presented with the nutrient formula allows slowing the transit of nutrients that are delivered later. Except for the start of feeding when transit can be too rapid because the inhibitory feedback from the composition has yet to be fully activated, once equilibrium is established, it is no longer logistically an issue of delivering the composition as a premeal although the physiologic principle is still the same.

Before dietary fats can be absorbed, the motor activities of the small intestine in the postprandial period must first move the output from the stomach to the appropriate absorptive sites of the small intestine. To achieve the goal of optimizing the movement of a substance through the small intestine, the temporal and spatial patterns of intestinal motility are specifically controlled by the nutrients of the luminal content.

Without wishing to be bound by any theory, it is presently believed that early in gastric emptying, before inhibitory feedback is activated, the load of fat entering the small intestine can be variable and dependent on the load of fat in the meal. Thus, while exposure to fat can be limited to the proximal small bowel after a small load, a larger load, by overwhelming more proximal absorptive sites, can spill further along the small bowel to expose the distal small bowel to fat. Thus, the response of the duodenum to fat limits the spread of fat so that more absorption can be completed in the proximal small intestine and less in the distal small intestine. Furthermore, since the speed of movement of luminal fat must decrease when more fat enters the duodenum, in order to avoid steatorrhea, intestinal transit is inhibited in a load-dependent fashion by fat. This precise regulation of intestinal transit occurs whether the region of exposure to fat is confined to the proximal gut or extended to the distal gut.

In accordance with the present invention it has been observed that inhibition of intestinal transit by fat depends on the load of fat entering the small intestine. More specifically, that intestinal transit is inhibited by fat in a load-dependent fashion whether the nutrient is confined to the proximal segment of the small bowel or allowed access to the whole gut.

As described above, the inventive technology can also operate by transmitting to and replicating at a second location in the upper gastrointestinal tract a serotonergic neural signal

originating at a first location in the proximal or distal gut of a mammal. For example, the first location can be in the proximal gut and the second location can be elsewhere in the proximal gut or in the distal gut. Or conversely, the first location can be in the distal gut and the second location can be elsewhere in the distal gut or in the proximal gut.

5 Employing this inventive technology to slow the rate of upper gastrointestinal transit, during and after a meal, nutrient absorption in the upper gastrointestinal tract is enhanced, depriving bacterial populations in the lower small intestine of nutrients. In response to luminal fat in the proximal or distal gut, a serotonin (5-HT)-mediated anti-peristaltic slowing response is normally present. Therefore, some embodiments of the method involve
10 increasing 5-HT in the gut wall by administering to the mammal and delivering to the proximal and/or distal gut, an active lipid, or serotonin, a serotonin agonist, or a serotonin re-uptake inhibitor.

 Alternatively, the active agent is PYY, or a PYY functional analog. PYY or the PYY analog activates the PYY-sensitive primary sensory neurons in response to fat or 5-HT.
15 Since the predominant neurotransmitter of the PYY-sensitive primary sensory neurons is calcitonin gene-related peptide (CGRP), in another embodiment, CGRP or a CGRP functional analog is the active agent.

 In other embodiments the point of action is an adrenergic efferent neural pathway, which conducts neural signals from one or more of the celiac, superior mesenteric, and
20 inferior mesenteric prevertebral ganglia, back to the enteric nervous system. The active agent is an adrenergic receptor (i.e., adrenoceptor) agonist to activate neural signal transmission to the efferent limb of the anti-peristaltic reflex response to luminal fat.

 Since adrenergic efferent neural pathway(s) from the prevertebral ganglia to the enteric nervous system stimulate serotonergic interneurons, which in turn stimulate
25 enteric opioid interneurons, in other embodiments of the method, the active agent is 5-HT, 5-HT receptor agonist, or a 5-HT re-uptake inhibitor to activate or enhance neural signal transmission at the level of the serotonergic interneurons.

 Alternatively, the active agent is an opioid receptor agonist to activate or enhance neural signal transmission via the opioid interneurons.

30 In accordance with the invention, pharmaceutically acceptable compositions containing the active agent can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups, elixirs or enteral formulas. Compositions intended for oral use

can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more other agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients can also be manufactured by known methods. The excipients used can be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. They can also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release. Other techniques for controlled release compositions, such as those described in the U.S. Pat. Nos. 4,193,985; and 4,690,822; 4,572,833 can be used in the formulation of the inventive pharmaceutically acceptable compositions.

In some cases, formulations for oral use can be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They can also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

In one embodiment of the present invention, the pharmaceutically acceptable composition is an enterically coated or a sustained release form that permits intestinal transit to be slowed for a prolonged period of time.

In an alternative aspect of the method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, after the presence of SIBO is detected in the human subject by suitable detection means, as described above, a pharmaceutically acceptable disinfectant composition is introduced into the lumen of the small intestine so as to contact the bacteria constituting the SIBO condition. The

disinfectant composition is introduced in an amount sufficient to inhibit the growth of the bacteria in the small intestine, thereby at least partially eradicating SIBO in the human subject.

Preferably, the pharmaceutically acceptable disinfectant composition consists essentially of hydrogen peroxide; a bismuth-containing compound or salt; or an iodine-containing compound or salt. The pharmaceutically acceptable disinfectant (i.e., bacteriocidal) composition can also contain other non-bacteriocidal ingredients, such as any suitable pharmaceutically acceptable carrier, excipient, emulsant, solvent, colorant, flavorant, and/or buffer, as described hereinabove. Formulations for oral or enteral delivery are useful, as described hereinabove with respect to known delivery modalities for active agents, e.g., tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups, elixirs or enteral formulas.

Embodiments of disinfectant or bacteriocidal compositions containing hydrogen peroxide are known for internal use in vertebrates (e.g., Ultradyne, Ultra Bio-Logics Inc., Montreal, Canada). Preferably, an aqueous solution of about 1% to about 3% (v/v) hydrogen peroxide is introduced orally or otherwise enterally to the lumen, most conveniently by ingestion.

Embodiments of disinfectant or bacteriocidal compositions containing bismuth compounds or salts are also known, for example, bismuth-2-3-dimercaptopropanol (BisBAL), bismuth thiols (e.g., bismuth-ethanedithiol), or bismuth-3,4-dimercaptotoluene (BisTOL), and in over the counter preparations, such as PeptoBismol. (See, e.g., Domenico, P. et al., Activity of Bismuth Thiols against Staphylococci and Staphylococcal biofilms, *Antimicrob. Agents Chemother.* 45(5):1417-21 [2001]).

Embodiments of disinfectant or bacteriocidal compositions containing iodine compounds or salts are also known, for example, povidone-iodine solutions.

In still another alternative aspect of the method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, after the presence of SIBO is detected in the human subject by suitable detection means, as described above, a pharmaceutically acceptable composition is administered to the subject. The pharmaceutically acceptable composition contains a stabilizer of mast cell membranes in the luminal wall of the small intestine, in an amount sufficient to inhibit a mast cell-mediated immune response in the human subject. This embodiment is a relatively aggressive treatment and is most useful in more severe or advanced SIBO, for example, as confirmed

by high intestinal permeability in the subject (see hereinabove). Suitable mast cell stabilizers include oxatamide or chromoglycate (potassium or sodium salts preferred). (e.g., Pacor, M.L. *et al.*, *Controlled study of oxatamide vs disodium chromoglycate for treating adverse reactions to food*, *Drugs Exp Clin Res* 18(3):119-23 [1992]; Stefanini, G.F. *et al.*, *Oral cromolyn sodium in comparison with elimination diet in the irritable bowel syndrome, diarrheic type*, Multicenter Study of 428 patients, *Scand. J. Gastroenterol.* 30(6):535-41 [1995]; Andre, F. *et al.*, *Digestive permeability to different-sized molecules and to sodium cromoglycate in food allergy*, *Allergy Proc.* 12(5):293-98 [1991]; Lunardi, C. *et al.*, *Double-blind cross-over trial of oral sodium cromoglycate in patients with irritable bowel syndrome due to food intolerance*, *Clin Exp Allergy* 21(5):569-72 [1991]; Burks, A.W. *et al.*, *Double-blind placebo-controlled trial of oral cromolyn in children with atopic dermatitis and documented food hypersensitivity*, *J. Allergy Clin. Immunol.* 81(2):417-23 [1988]).

After the SIBO condition is at least partially eradicated, typically within a couple of weeks, there is an improvement in the symptom(s) of irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, chronic pelvic pain syndrome, autism, impaired mentation, impaired memory, depression, ADHD, an autoimmune disease, or *Crohn's* disease. It is a benefit of the inventive treatment method that after treatment, subjects routinely report feeling better than they have felt in years.

The inventive method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, as described above, can be optionally combined, simultaneously or in sequence, with other suitable methods of at least partially eradicating small intestinal bacterial overgrowth, such as the following.

For example, at least partially eradicating the bacterial overgrowth is accomplished by administering an antimicrobial agent, including but not limited to a natural, synthetic, or semi-synthetic antibiotic agent. For example, a course of antibiotics such as, but not limited to, neomycin, metronidazole, teicoplanin, doxycycline, tetracycline, ciprofloxacin, augmentin, cephalexin (e.g., Keflex), penicillin, ampicillin, kanamycin, rifamycin, rifaximin, or vancomycin, which may be administered orally, intravenously, or rectally. (R.K. Cleary [1998]; C.P. Kelly and J.T. LaMont, *Clostridium difficile* infection, *Annu. Rev. Med.* 49:375-90 [1998]; C.M. Reinke and C.R. Messick, *Update on Clostridium difficile-induced colitis, Part 2*, *Am. J. Hosp. Pharm.* 51(15):1892-1901 [1994]).

Alternatively, an antimicrobial chemotherapeutic agent, such as a 4- or 5-aminosalicylate compound is used to at least partially eradicate the SIBO condition. These

can be formulated for ingestive, colonic, or topical non-systemic delivery systems or for any systemic delivery systems. Commercially available preparations include 4-(p)-aminosalicylic acid (i.e., 4-ASA or para-aminosalicylic acid) or 4-(p)-aminosalicylate sodium salt (e.g., Nemasol-Sodium or Tubasal). 5-Aminosalicylates have antimicrobial, as well as anti-inflammatory properties (H. Lin and M. Pimentel, Abstract G3452 at Digestive Disease Week, 100th Annual Meeting of the AGA, Orlando, FL [1999]), in useful preparations including 5-aminosalicylic acid (i.e., 5-ASA, mesalamine, or mesalazine) and conjugated derivatives thereof, available in various pharmaceutical preparations such as Asacol, Rowasa, Claversal, Pentasa, Salofalk, Dipentum (olsalazine), Azulfidine (SAZ; sulphasalazine), ipsalazine, salicylazobenzoic acid, balsalazide, or conjugated bile acids, such as ursodeoxycholic acid-5-aminosalicylic acid, and others.

Another preferred method of at least partially eradicating small intestinal bacterial overgrowth, particularly useful when a subject does not respond well to oral or intravenous antibiotics or other antimicrobial agents alone, is administering an intestinal lavage or enema, for example, small bowel irrigation with a balanced hypertonic electrolyte solution, such as Go-lytely or fleet phosphosoda preparations. The lavage or enema solution is optionally combined with one or more antibiotic(s) or other antimicrobial agent(s). (E.g., J.A. Vanderhoof *et al.*, *Treatment strategies for small bowel bacterial overgrowth in short bowel syndrome*, J. Pediatr. Gastroenterol. Nutr. 27(2):155-60 [1998])

Another preferred method of at least partially eradicating small intestinal bacterial overgrowth employs a probiotic agent, for example, an inoculum of a lactic acid bacterium or bifidobacterium. (A.S. Naidu *et al.*, *Probiotic spectra of lactic acid bacteria*, Crit. Rev. Food Sci. Nutr. 39(1):13-126 [1999]; J.A. Vanderhoof *et al.* [1998]; G.W. Tannock, *Probiotic properties of lactic acid bacteria: plenty of scope for R & D*, Trends Biotechnol. 15(7):270-74 [1997]; S. Salminen *et al.*, *Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges*, Antonie Van Leeuwenhoek 70(2-4):347-58 [1997]). The inoculum is delivered in a pharmaceutically acceptable ingestible formulation, such as in a capsule, or for some subjects, consuming a food supplemented with the inoculum is effective, for example a milk, yoghurt, cheese, meat or other fermentable food preparation. Useful probiotic agents include *Bifidobacterium sp.* or *Lactobacillus* species or strains, e.g., *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. reuteri*, *L. paracasei* subsp. *paracasei*, or *L. casei* Shirota, (P. Kontula *et al.*, *The effect of lactose derivatives on intestinal lactic acid bacteria*, J. Dairy Sci. 82(2):249-56 [1999]; M. Alander *et al.*, *The effect*

of probiotic strains on the microbiota of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), *Int. J. Food Microbiol.* 46(1):71-79 [1999]; S. Spanhaak *et al.*, *The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans*, *Eur. J. Clin. Nutr.* 52(12):899-907 [1998];
5 W.P. Charteris *et al.*, *Antibiotic susceptibility of potentially probiotic Lactobacillus species*, *J. Food Prot.* 61(12):1636-43 [1998]; B.W. Wolf *et al.*, *Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus*, *Food Chem. Toxicol.* 36(12):1085-94 [1998]; G. Gardiner *et al.*, *Development of a probiotic cheddar cheese containing human-derived Lactobacillus paracasei strains*, *Appl. Environ.*
10 *Microbiol.* 64(6):2192-99 [1998]; T. Sameshima *et al.*, *Effect of intestinal Lactobacillus starter cultures on the behaviour of Staphylococcus aureus in fermented sausage*, *Int. J. Food Microbiol.* 41(1):1-7 [1998]).

Optionally, after at least partial eradication of small intestinal bacterial overgrowth, use of antimicrobial agents or probiotic agents can be continued to prevent further
15 development or relapse of SIBO.

Another preferred method of at least partially eradicating small intestinal bacterial overgrowth is by normalizing or increasing phase III interdigestive intestinal motility between meals with any of several modalities to at least partially eradicate the bacterial overgrowth, for example, by suitably modifying the subject's diet to increase small intestinal
20 motility to a normal level (e.g., by increasing dietary fiber), or by administration of a chemical prokinetic agent to the subject, including bile acid replacement therapy when this is indicated by low or otherwise deficient bile acid production in the subject.

For purposes of the present invention, a prokinetic agent is any chemical that causes an increase in phase III interdigestive motility of a human subject's intestinal tract.
25 Increasing intestinal motility, for example, by administration of a chemical prokinetic agent, prevents relapse of the SIBO condition, which otherwise typically recurs within about two months, due to continuing intestinal dysmotility. The prokinetic agent causes an increase in phase III interdigestive motility of the human subject's intestinal tract, thus preventing a recurrence of the bacterial overgrowth. Continued administration of a prokinetic agent to
30 enhance a subject's phase III interdigestive motility can extend for an indefinite period as needed to prevent relapse of the SIBO condition.

Preferably, the prokinetic agent is a known prokinetic peptide, such as motilin, or functional analog thereof, such as a macrolide compound, for example, erythromycin

(50mg/day to 2000mg/day in divided doses orally or I.V. in divided doses), or azithromycin (250-1000 mg/day orally).

However, a bile acid, or a bile salt derived therefrom, is another preferred prokinetic agent for inducing or increasing phase III interdigestive motility. (E.P. DiMagno, *Regulation of interdigestive gastrointestinal motility and secretion*, Digestion 58 Suppl. 1:53-55 [1997]; V.B. Nieuwenhuijs *et al.*, *Disrupted bile flow affects interdigestive small bowel motility in rats*, Surgery 122(3):600-08 [1997]; P.M. Hellstrom *et al.*, *Role of bile in regulation of gut motility*, J. Intern. Med. 237(4):395-402 [1995]; V. Plourde *et al.*, *Interdigestive intestinal motility in dogs with chronic exclusion of bile from the digestive tract*, Can. J. Physiol. Pharmacol. 65(12):2493-96 [1987]). Useful bile acids include ursodeoxycholic acid and chenodeoxycholic acid; useful bile salts include sodium or potassium salts of ursodeoxycholate or chenodeoxycholate, or derivatives thereof.

A compound with cholinergic activity, such as cisapride (i.e., Propulsid ; 1 to 20 mg, one to four times per day orally or I.V.), is also preferred as a prokinetic agent for inducing or increasing phase III interdigestive motility. Cisapride is particularly effective in alleviating or improving hyperalgesia related to SIBO or associated with disorders caused by SIBO, such as IBS, fibromyalgia, or *Crohn's* disease.

A dopamine antagonist, such as metoclopramide (1-10 mg four to six times per day orally or I.V.), domperidone (10 mg, one to four times per day orally), or bethanechol (5 mg/day to 50 mg every 3-4 hours orally; 5-10 mg four times daily subcutaneously), is another preferred prokinetic agent for inducing or increasing phase III interdigestive motility. Dopamine antagonists, such as domperidone, are particularly effective in alleviating or improving hyperalgesia related to SIBO or associated with disorders caused by SIBO, such as IBS, fibromyalgia, or *Crohn's* disease.

Also preferred is a nitric oxide altering agent, such as nitroglycerin, , nomega-nitro-L-arginine methylester (L-NAME), N-monomethyl-L-arginine (L-NMMA), or a 5-hydroxytryptamine (HT or serotonin) receptor antagonist, such as ondansetron (2-4 mg up to every 4-8 hours I.V.; pediatric 0.1mg/kg/day) or alosetron. The 5-HT receptor antagonists, such as ondansetron and alosetron, are particularly effective in improving hyperalgesia related to SIBO, or associated with disorders caused by SIBO, such as IBS, fibromyalgia, or *Crohn's* disease.

An antihistamine, such as promethazine (oral or I.V. 12.5 mg/day to 25 mg every four hours orally or I.V.), meclizine (oral 50 mg/day to 100 mg four times per day), or other

antihistamines, except ranitidine (Zantac), famotidine, and nizatidine, are also preferred as prokinetic agents for inducing or increasing phase III interdigestive motility.

Also preferred are neuroleptic agents, including prochlorperazine (2.5 mg/day to 10 mg every three hours orally; 25 mg twice daily rectally; 5 mg/day to 10 mg every three hours, not to exceed 240 mg/day intramuscularly; 2.5 mg/day to 10 mg every four hours I.V.), chlorpromazine (0.25 mg/lb. up to every four hours [5-400 mg/day] orally; 0.5 mg/lb. up to every 6 hours rectally; intramuscular 0.25/lb. every six hours, not to exceed 75/mg/day), or haloperidol (oral 5-10 mg/day orally; 0.5-10 mg/day I.V.). Also useful as a prokinetic agent, for purposes of the present invention, is a kappa agonist, such as fedotozine (1-30 mg/day), but not excluding other opiate agonists. The opiate (opioid) agonists, such as fedotozine, are particularly effective in alleviating or improving hyperalgesia related to SIBO or associated with disorders caused by SIBO, such as IBS, fibromyalgia, or *Crohn's* disease.

The preceding are merely illustrative of the suitable means by which small intestinal bacterial overgrowth is at least partially eradicated by treatment in accordance or in combination with the inventive methods. These means can be used separately, or in combination, by the practitioner as suits the needs of an individual human subject.

Optionally, treating further includes administering to the human subject an anti-inflammatory cytokine or an agonist thereof, substantially simultaneously with or after at least partially eradicating the bacterial overgrowth of the small intestine, to accelerate or further improve the symptom(s) of irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, depression, ADHD, or an autoimmune disease, or *Crohn's* disease. Useful anti-inflammatory cytokines include human IL-4, IL-10, IL-11, or TGF- β , derived from a human source or a transgenic non-human source expressing a human gene. The anti-inflammatory cytokine is preferably injected or infused intravenously or subcutaneously.

Optionally, when the suspected diagnosis is irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, depression, ADHD, or an autoimmune disease, such as multiple sclerosis or systemic lupus erythematosus, symptoms are improved by administering an antagonist of a pro-inflammatory cytokine or an antibody that specifically binds a pro-inflammatory cytokine. The antagonist or antibody is administered to the human subject substantially simultaneously with or after treatment to at least partially eradicate the bacterial overgrowth. The antagonist or antibody is one that binds to a pro-inflammatory cytokine or antagonizes the activity or receptor binding of a pro-inflammatory cytokine. Pro-inflammatory cytokines include TNF- α , IL-1 α , IL-1 β , IL-6, IL-8, IL-12, or LIF. The

cytokine antagonist or antibody is preferably derived from a human source or is a chimeric protein having a human protein constituent. The cytokine antagonist or antibody is preferably delivered to the human subject by intravenous infusion.

Optionally, the method of treating irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, depression, attention deficit/hyperactivity disorder, an autoimmune disease, or *Crohn's* disease, further comprises administering an agent that modifies afferent neural feedback or sensory perception. This is particularly useful when, after at least partial eradication of SIBO, the subject experiences residual symptoms of hyperalgesia related to SIBO or associated with a disorder caused by SIBO, such as IBS, fibromyalgia, or *Crohn's* disease. Agents that modify afferent neural feedback or sensory perception include 5-HT receptor antagonists, such as ondansetron and alosetron; opiate agonists, such as fentanyl; peppermint oil; cisapride; a dopamine antagonist, such as domperidone; an antidepressant agent; an anxiolytic agent; or a combination of any of these. Useful antidepressant agents include tricyclic antidepressants, such as amitriptyline (Elavil); tetracyclic antidepressants, such as maprotiline; serotonin re-uptake inhibitors, such as fluoxetine (Prozac) or sertraline (Zoloft); monoamine oxidase inhibitors, such as phenelzine; and miscellaneous antidepressants, such as trazodone, venlafaxine, mirtazapine, nefazodone, or bupropion (Wellbutrin). Typically, useful antidepressant agents are available in hydrochloride, sulfated, or other conjugated forms, and all of these conjugated forms are included among the useful antidepressant agents. Useful anxiolytic (anti-anxiety) agents include benzodiazepine compounds, such as Librium, Atavine, Xanax, Valium, Tranxene, and Serax, or other anxiolytic agents such as Paxil.

Eradication of the bacterial overgrowth is determined by detection methods described above, particularly in comparison with recorded results from pre-treatment detection. After at least partially eradicating the bacterial overgrowth, in accordance with the present method, the symptom(s) of irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, depression, ADHD, an autoimmune disease, or *Crohn's* disease are improved. Improvement in a symptom(s) is typically determined by self-reporting by the human subject, for example by VAS scoring or other questionnaire. Improvement in academic, professional, or social functioning, e.g., in cases of ADHD or depression can also be reported by others or can be observed by the clinician. Improvement (increase) in pain threshold, e.g., in subjects diagnosed with fibromyalgia, can be measured digitally, for example, by tender point count, or mechanically, for example, by dolorimetry. (F. Wolfe *et al.*, *Aspects of Fibromyalgia in*

the General Population: Sex, Pain Threshold, and Fibromyalgia Symptoms, J. Rheumatol. 22:151-56 [1995]). Improvement in visceral hypersensitivity or hyperalgesia can be measured by balloon distension of the gut, for example, by using an electronic barostat. (B.D. Nabiloff *et al.*, *Evidence for two distinct perceptual alterations in irritable bowel syndrome*, Gut 41:505-12 [1997]). Some improvement(s) in symptoms, for example systemic lupus erythematosus symptoms, such as rashes, photosensitivity, oral ulcers, arthritis, serositis, or improvements in the condition of blood, kidney or nervous system, can be determined by clinical observation and measurement.

10 The present invention also relates to a kit for the diagnosis of SIBO or a SIBO-caused condition. The kit comprises at least one breath sampling container, a pre-measured amount of a substrate, and instructions for a user in detecting the presence or absence of SIBO by determining the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by the human subject, after the human subject has ingested a controlled quantity of the substrate. The present kit is useful for practicing the inventive method of detecting SIBO in a human subject, as described hereinabove.

15 The kit is a ready assemblage of materials or components for facilitating the detection of small intestinal bacterial overgrowth, in accordance with the present invention. The kit includes suitable storage means for containing the other components of the kit. The kit includes at least one, and most preferably multiple, air-tight breath sampling container(s), such as a bag, cylinder, or bottle, and at least one pre-measured amount of a substrate, which is preferably an isotope-labeled substrate or substrate that is poorly digestible by a human. Preferably the substrate is a sugar, such as lactulose (e.g., 10-20 g units) or xylose, or a sugar, such as glucose (e.g., 75-80 g units), lactose, or sucrose, for measuring breath hydrogen, methane, and at least one sulfur-containing gas, such as hydrogen sulfide, a sulfhydryl compound, methanethiol, dimethylsulfide, dimethyl disulfide, an allyl methyl sulfide, an allyl methyl sulfide, an allyl methyl disulfide, an allyl disulfide, an allyl mercaptan, or a methylmercaptan.

25 The present kit also contains instructions for a user in how to use the kit to detect small intestinal bacterial overgrowth (SIBO) or to corroborate a suspected diagnosis of irritable bowel syndrome, fibromyalgia, chronic fatigue syndrome, chronic pelvic pain syndrome, autism, impaired mentation, impaired memory, depression, ADHD, an autoimmune disease, or *Crohn's* disease, in accordance with the present methods.

Optionally, the kit also contains compositions useful for at least partially eradicating SIBO, as described above.

The components assembled in the kits of the present invention are provided to the practitioner stored in any convenient and suitable way that preserves their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

The foregoing descriptions for the methods and kits of the present invention are illustrative and by no means exhaustive. The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Example 1: Composition of the Database.

Data were assembled from 202 human subjects from the Cedars-Sinai Medical Center GI Motility Program who completed an extensive questionnaire of health history. These patients were all referred for lactulose breath hydrogen testing (LBHT) by more than 30 private gastroenterologists. These patients were selected by their gastroenterologists to undergo breath testing, because they had symptoms compatible with SIBO. However, the questionnaire focused on general risk factors, associated conditions, and symptoms found in these patients and not specifically the incidence of SIBO. After antibiotic therapy, 59 subjects actually returned for a follow up LBHT and a follow-up questionnaire. This likely resulted in an underestimate of responsiveness to treatment, since only those who failed to respond adequately were likely to return to assess eradication of SIBO.

Example 2: Breath Hydrogen Testing.

Subjects were tested after an overnight fast. At time zero, each subject swallowed 15 ml of Chronulac formula, delivering 10 g of lactulose; every 5-20 min thereafter, for 2-4 hours, a 50 cm³ end-expiratory breath sample was taken with an airtight sampling bag. Each breath sample was then analyzed for hydrogen content with a gas chromatograph (Quintron Model DP, Quintron Instrument Co., Division of E.F. Brewer Co, Menomonee Falls, WI 53051), standardized using a QuinGas standard as instructed by the manufacturer. Hydrogen peaks were plotted before and after an antimicrobial treatment regimen for comparison. The normal range for the second hydrogen peak was 0 to 20 ppm.

Example 3: Diagnosis and Antibiotic Treatment of Irritable Bowel Syndrome.

The two hundred-two (202) human subjects were assessed for SIBO with LBHT. Of the 202 subjects in the database, 95 claimed to have been given a diagnosis of IBS. In addition, a symptom questionnaire was used to determine whether these subjects fulfilled Rome criteria for IBS, and four of the subjects failed to meet the Rome criteria. *Crohn's* disease was present in 14 of the subjects and four had a history of ulcerative colitis. After these 22 subjects were excluded, 73 subjects remained.

Among the 107 subjects who stated that they had not previously been given a diagnosis of IBS, 78 met Rome criteria. After the 21 who had *Crohn's* disease, five who had ulcerative colitis and one with short bowel transit were excluded, 51 subjects remained. Data gathered from these subjects were pooled with data from the previous 73 subjects with suspected IBS, yielding a total of 124 of the original 202 (61%) subjects with a suspected diagnosis of IBS.

Of the 124, 92 (74%) were positive for SIBO. However, of the 32 subjects meeting the Rome criteria, who were negative for SIBO, 14 had been treated with antibiotics within 3 months prior to LBHT. Therefore, the incidence of SIBO among the 110 untreated subjects was 92 (84%), showing a strong association between a suspected diagnosis of IBS and the presence of SIBO. After neomycin treatment (500 mg twice daily for ten days), 23 of these 92 returned for follow-up testing. On a visual analog scores (VAS), subjects were asked to rate their degree of post-treatment improvement. These 23 subjects reported a $60 \pm 31\%$ improvement, although 17 had only partial eradication of SIBO, based on their LBHT results. (Figure 1).

There was a likely selection bias in the database due to the fact that subjects were referred for LBHT, because their physicians suspected they had SIBO. To correct for this bias, a pilot study was also conducted looking at the incidence of bacterial overgrowth in patients with IBS. All patients between the ages of 18 and 65 referred to the Cedars-Sinai GI Motility Program who met Rome criteria for IBS, and who had had a previous upper GI (small bowel) with follow-through (i.e., barium or Gastrografin imaging analysis) ruling out *Crohn's* disease and ulcerative colitis, were asked to present to the GI motility laboratory for LBHT. Eight human subjects with a suspected diagnosis of IBS, based on the Rome criteria, were tested for SIBO, using LBHT as described in Example 2. Seven of these patients (87.5%) were found to have SIBO based on hydrogen peaks in a range of 80-250 ppm of hydrogen. Six of the 7 subjects testing positive for SIBO returned approximately 10

days after completion of a 10 day course of neomycin as described above. Neomycin treatment completely eradicated the SIBO in each of the six subjects, based on post-treatment breath hydrogen peaks in the normal range of 0-20 ppm. The six subjects reported an average improvement in their IBS symptoms of $65 \pm 28\%$ (Range: 20-100%) on VAS scoring.

5 Figure 2 shows VAS for the six subjects, based on a scale of 0-5, with 0 implying no pain and 5 the most pain of life-time. It is clear from these results that at least partial eradication of bacterial overgrowth results in an improvement in gastrointestinal symptoms including bloating, gas, diarrhea, abdominal pain, sensation of incomplete evacuation and even constipation, associated with IBS. Additionally, significant extraintestinal symptoms of IBS,
10 such as joint pain and fatigue, were also substantially improved, and the degree of improvement was greater in subjects who had complete eradication of SIBO.

Comparison of efficacies of various antibiotic regimes for treating SIBO. Subjects referred to the Cedars-Sinai GI Motility Program for a lactulose breath hydrogen test
15 (LBHT) to assess SIBO were entered into a database. Those that tested positive for SIBO were given antibiotic treatment by their referring physician and in some cases, returned for a follow-up LBHT to assess eradication of SIBO. During the follow-up LBHT, subjects were asked which antibiotic they were given to treat their SIBO. The eradication rate of each antibiotic was evaluated.

20 Of the 771 subjects in the database, 561 (73%) tested positive for SIBO. Of the 170 subjects who returned for a follow-up LBHT, 65 subjects were excluded because they did not specify or could not remember which antibiotic they took. Based on the remaining 105 subjects, neomycin, augmentin, and ciprofloxacin were the most commonly prescribed, with neomycin being most successful. (See Table 1 below).
25 Flagyl was a relatively poor choice by itself. None of the commonly used antibiotics was universally successful in eradicating overgrowth. Thus, Table 1 shows that, while a number of antibiotics are able to eradicate SIBO, neomycin was most effective.

Table 1. Comparison of efficacies of various antibiotic regimes for treating SIBO

| | Number of Patients SIBO Eradicated | Total Number | % Patients with SIBO Eradicated |
|----------|---------------------------------------|--------------|---------------------------------------|
| Neomycin | 42 | 76 | 55 |
| Flagyl | 2 | 8 | 25 |

| | | | |
|-------------------------------|---|---|----|
| Ciprofloxacin | 3 | 6 | 50 |
| Augmentin | 2 | 4 | 50 |
| Flagyl + Ciprofloxacin | 1 | 2 | —* |
| Tetracycline | 2 | 2 | —* |
| Doxycycline | 1 | 1 | —* |
| Trovan | 0 | 1 | —* |
| Neomycin/Biaxin + Amoxicillin | 1 | 1 | —* |
| Neomycin + Ciprofloxacin | 1 | 1 | —* |
| Tetracycline + Flagyl | 1 | 1 | —* |
| Neomycin + Flagyl | 0 | 1 | —* |
| Biaxin | 0 | 1 | —* |

*Too few subjects to determine percent success.

5 Prevalence of SIBO in normal controls: The prevalence of SIBO in IBS compared to normal controls was determined as defined by the lactulose hydrogen breath test. Fifty-seven IBS subjects enrolled in a double blind placebo controlled trial and 9 normal controls underwent a lactulose breath hydrogen test (LBHT) to diagnose SIBO. IBS subjects had to meet Rome I criteria. Control subjects had to have none of the Rome I criteria, based on telephone or in-person interviews. SIBO was defined as a greater than 20 ppm rise in H₂ concentration during the first 90 minutes of lactulose breath hydrogen testing. The prevalence of SIBO in IBS subjects and controls was compared using Chi-square.

10 Of the 57 IBS subjects, 41 (72%) had SIBO. Of the 9 normal controls, only 1 subject (11%) had SIBO ($\chi^2=9.9$, OR=20.5, CI:2.2-481.8, $p<0.01$). These results confirm the association between IBS and SIBO as there is a much higher prevalence of SIBO in IBS compared to normal controls.

Example 4: Diagnosis and Treatment of Fibromyalgia and Chronic Fatigue Syndrome.

20 Fibromyalgia: Of the 202 patients in the database, 37 (18%) had a suspected diagnosis of fibromyalgia. Of these 37, 28 tested positive for SIBO. However, of the nine who tested negative for SIBO, six had taken antibiotics within the preceding 3 months, and were excluded. Therefore, 28 out of 30 (93%) of subjects with suspected fibromyalgia had SIBO, demonstrating a strong association between a suspected diagnosis of fibromyalgia and the presence of SIBO.

25 After neomycin treatment (500 mg twice daily, 10-day course), ten of these 28 subjects returned, and post-treatment LBHT confirmed that SIBO had been at least partially

eradicated. These ten subjects reported a $63 \pm 19\%$ overall improvement in their symptoms by VAS scoring. Figure 3 compares the VAS scores for various symptoms reported by the subjects with a suspected diagnosis of fibromyalgia before and after neomycin treatment.

Symptoms included bloating, gas, diarrhea, joint pain and fatigue to treatment. Subjects were asked to identify the symptom most improved. Five subjects reported that pain was the most improved; three subjects reported that the level of fatigue was most improved, and two others reported that their abdominal complaints improved the most. There was a negative correlation between the degree of improvement in the VAS scoring and the amount of residual hydrogen peak seen in LBHT. (Pearson=-0.689, $p=0.02$; Figure 4).

Subsequently, forty-six human subjects with FM (ACR criteria) entered a double blind randomized placebo controlled trial. Each subject underwent LBHT, a tender point examination and completed a questionnaire at the initial (baseline) and at every subsequent visit. Subjects were randomized to receive neomycin (500 mg twice daily in liquid form) or a matched placebo, for 10 days. After completion of this treatment, subjects with persistent SIBO received antibiotics (open label) until at least partially eradication was confirmed by LBHT. T-test was used to compare the symptom scores of patients whose SIBO condition was at least partially eradicated with those whose SIBO was not at least partially eradicated.

Forty-two of the 46 FM patients (91.3%) were found to have SIBO. Six out of 20 patients (30%) in the neomycin group achieved complete at least partially eradication in the blinded arm. Only 6 subjects showed no difference in the symptom score before and after the 10 d treatment. Twenty-eight subjects went on to open label treatment with 17 (60.7%) achieving complete at least partially eradication of SIBO. When symptom scores after at least partially eradication of SIBO on double blind or open treatment were compared to baseline, there was significant improvement in Tender Points, Tender Point Score, Hamilton Depression Scale, Fibromyalgia Impact Questionnaire (FIQ), Beck Depression Scale, Health Assessment Questionnaire (HAQ), VAS-Pain, VAS-Memory/Concentration and IBS-Quality of Life (QOL). (Initial data in Table 1a). These results confirm that SIBO is associated with fibromyalgia, and that at least partially eradication of SIBO improves symptoms in fibromyalgia.

Table 1a. Selected Symptom Scores Double Blind Randomized Placebo Controlled Trial with Subjects Diagnosed with Fibromyalgia.

| | | SIBO eradicated (n=25) | | | SIBO not eradicated (p=15) | | eradicated vs. not eradicated |
|-----------------------|-----------|------------------------------|---------|-----------|----------------------------------|---------|-------------------------------------|
| Observation | Baseline | eradicated | P-value | Baseline | eradicated | P-value | P-value |
| Tender Points (TP) | 13.3±2.9 | 10.3±4.2 | 0.01 | 13.6±2.0 | 12.1±4.1 | NS | NS |
| TP Score | 20.3±7.0 | 15.0±9.1 | 0.01 | 23.7±8.0 | 19.9±9.7 | NS | NS |
| FIQ | 66.8±18.2 | 49.5±17.7 | 0.0001 | 72.7±19.9 | 64.1±20.9 | 0.04 | 0.02 |
| VAS-pain(mm) | 80.7±22.7 | 52.4±28.5 | 0.00005 | 87.5±19.6 | 76.2±25.2 | NS | 0.01 |
| HAQ | 42.4±10.5 | 37.7±10.1 | 0.005 | 45.1±11.2 | 43.9±12.1 | NS | NS |

Chronic Fatigue Syndrome: Thirty of 202 subjects in the database (15.9%) had received a diagnosis of chronic fatigue syndrome. Of these 30 subjects, 21 (70%) had SIBO as indicated by LBHT, but four out of the nine without SIBO had recently taken antibiotics. Therefore, the prevalence of SIBO was 21 out of 26 (81%) subjects with a diagnosis of CFS.

After treatment with neomycin (500 mg twice daily, 10-day course), nine of the 21 subjects diagnosed with CFS, returned for follow-up LBHT and questionnaire. LBHT showed that all nine subjects experienced at least partially eradication of SIBO, and important symptoms of CFS were substantially improved after treatment. (Table 2).

Table 2. VAS scores by CFS patients reporting before and after anti-biotic treatment.

| Symptom | Before Antibiotic | After Antibiotic | P-value |
|----------|-------------------|------------------|---------|
| Bloating | 4.3 ± 1.0 | 2.3 ± 1.7 | 0.002 |
| Fatigue | 4.6 ± 1.0 | 3.5 ± 1.4 | 0.02 |

Example 5: Autoimmune Diseases, Depression, ADHD, Autism, Mentation and Memory.

SLE. Fifteen of the 202 (7.4%) subjects in the database had been diagnosed with SLE. Of these 15 subjects, 13 (87%) had bacterial overgrowth, as indicated by LBHT. Four of the 15 subjects with SLE returned for follow-up LBHT and questionnaire after treatment with neomycin (500 mg twice daily for 10 days). LBHT results for these four were negative for SIBO, and other significant symptoms were significantly improved after treatment. (Table 3).

Table 3. VAS scores by SLE patients reporting before and after anti-biotic treatment.

| Symptom | Before Antibiotic | After Antibiotic | P-value |
|-------------|-------------------|------------------|---------|
| Bloating | 3.0 ± 2.0 | 1.3 ± 1.3 | 0.1 |
| Joint Pains | 2.5 ± 1.5 | 0.5 ± 0.6 | 0.04 |
| Gas | 3.3 ± 1.7 | 1.9 ± 1.7 | 0.3 |
| Fatigue | 4.6 ± 1.0 | 3.5 ± 1.4 | 0.3 |

Multiple Sclerosis: A 22-year-old female who presented with a history of multiple sclerosis symptoms and with plaques demonstrated on MRI imaging. A suspected diagnosis of multiple sclerosis had been made by a neurologist was based on various neuropathies of the peripheral nervous system, including numbness, tingling, and weakness in the lower extremities, but this subject also had associated bloating, gas, distension and alteration in bowel habits. The subject also complained of a significant fatigue and nausea. The subject underwent LBHT, which detected SIBO. She was subsequently treated with neomycin (500 mg twice daily for 10 days), which at least partially eradicated the bacterial overgrowth. This was followed by complete resolution of her nausea, fatigue, bloating, gas distension and alteration in bowel habits. In addition, the subject showed dramatic improvement and resolution of her neuropathies. She no longer had numbness or tingling in the hands or feet and was functioning quite well. Approximately 6-8 weeks after this initial response, the patient had a relapse of her symptoms, including bloating, gas, distension and neuropathy. She had a repeat LBHT that confirmed a recurrence of SIBO. Upon re-treatment with neomycin (500 mg twice daily for 10 days), she once again experienced complete resolution of her symptoms.

Depression: A 73-year-old female presented with bloating, gas, abdominal distention, and cramping for a period of 3 years prior to LBHT. Symptoms of depression first appeared concurrently with the first appearance of bowel symptoms, and were serious enough that psychiatric hospitalization had been considered by her attending psychiatrist. The subject reported feeling very depressed and was convinced that life was not worth living. The subject's LBHT indicated the presence of a SIBO condition. After treatment with neomycin (500 mg twice daily for 10 days), the subject stated that she felt 100% better. She reported that her depression was completely resolved and that her energy was back to normal. In addition, her bowel symptoms were also completely improved. The subject had been prescribed eight different anti-depressant medications, all of which were discontinued as a result of her improvement.

ADHD: A 13 year-old female was brought in by her mother with a suspected diagnosis of attention deficit/hyperactivity disorder (AD type), made by a pediatrician. Concurrently, she also had significant bloating, gas and some alteration in bowel habits. She had initially been referred for diagnosis by her teachers and school counselors, because she had been having difficulty performing in school for the previous two to three years, after having previously been a very good student. Prior to the detection of SIBO, the subject had been treated with multiple pharmacologic agents for depression, including amitryptiline, with no noticeable improvement in her symptoms.

The subject underwent LBHT that demonstrated the presence of SIBO. The subject was treated with neomycin (500 mg twice daily for 10 days) and after complete at least partially eradication of the bacterial overgrowth, she had resolution of her bowel symptoms. Additionally, she started to get A averages in school again after being in the C range. She was able to concentrate better, and her teachers noticed a difference in her focus and attitude. Approximately two months later the subject had a relapse in her attention problem which was concurrent with a recurrence of the bacterial overgrowth, as detected by LBHT. After repeat treatment with neomycin (500 mg twice daily for 10 days), the subject again responded with improved concentration and resolution of bowel symptoms.

Autism: The patient was a 6-year-old female with a history of autism after having failed development after the age of one year. Before treatment, the patient was categorized as

having a developmental age of 15 months. She also complained of abdominal distension, gas, bloating and altered bowel habits. The patient was treated with Augmentin (500 mg twice a day for ten days), which resulted in a substantial improvement in bowel habits altogether. The bloating, gas, distension and diarrhea resolved. In addition, there were some positive concentration and behavioral changes. The patient was more responsive and cognitively appreciative of her parents' wishes, and there was some advancement in intellectual behavior. For example, after treatment she was able to tolerate clothing and had improved concentration.

- 10 Memory/Mentation/Concentration: The patient was a 72-year-old female with a history of chronic intestinal complaints over several years. She experienced altered bowel habits with alternating diarrhea and constipation with bloating, gas, distension and abdominal pain. Also, she had been diagnosed by several psychiatrists as having psychiatric problems due to decreased mentation from mild senility, and she contemplated psychiatric hospitalization.

15

SIBO was detected in this patient by LBHT. A subsequent course of antibiotics completely eradicated the SIBO condition, and she returned to report joyfully that she no longer needed the psychotropic medications that she had been prescribed, because she feels completely normal, including her bowels. She is now able to drive a car again, which was previously prevented from doing due to her impaired memory and difficulty in concentrating on the road. Treatment of her SIBO condition (neomycin, 500 mg twice a day for ten days) has produced a dramatic improvement in her quality of life.

20

Example 6: Diagnosis and Treatment of Crohn's disease.

25

Of the 202 subjects in the database, 39 (19%) had a suspected diagnosis of Crohn's disease. Of these 39, eight demonstrated short bowel transit and one subject produced neither hydrogen nor methane in LBHT; these nine were excluded. Of the 30 remaining subjects, 22 had SIBO. However, of the eight subjects who had a negative LBHT result, five had been treated with antibiotics within the preceding 3 months. If these subjects are excluded, 22 out of 25 (88%) subjects with a suspected diagnosis of Crohn's disease had SIBO, which shows a strong association between a suspected diagnosis of Crohn's disease and the presence of SIBO.

30

Of the 22 patients testing positive for the presence of SIBO, nine returned after neomycin treatment (10-day course of 500 mg twice/daily) for LBHT, which showed at least partially eradication of SIBO. These nine patients reported a $57 \pm 32\%$ ($n=8$ because one patient failed to report percent improvement) overall improvement in their symptoms by VAS. If these subjects remained positive after antibiotic treatment with neomycin, metronidazole (Flagyl), or ciprofloxacin, their improvement was only $20 \pm 0\%$ as opposed to $69 \pm 27\%$ if the breath test was negative ($p<0.05$). Figure 5 shows a dramatic improvement in the patients symptoms after treatment. There was an especially notable reduction in bloody stools, diarrhea and fatigue.

As with the subjects with fibromyalgia, there was a negative correlation between the degree of improvement in the VAS scoring and the amount of residual hydrogen production (Pearson=-0.787, $p=0.02$; Figure 6).

To correct for selection bias, a pilot study was conducted to determine the incidence of SIBO in subjects who had received a suspected diagnosis of Crohn's disease at Cedars-Sinai Medical Center's IBD Center within the preceding three months. Six of these subjects underwent LBHT, of whom five (83%) were positive for SIBO.

Two of the six subjects returned for follow-up after antibiotic therapy (10-day course of neomycin). Post-treatment LBHTs showed that SIBO had been completely at least partially eradicated in both subjects. They reported, respectively, a 60% and 80% overall improvement in their symptoms. This improvement was stated to include substantial reduction in diarrhea, gas and bloating.

Example 7: Response Stratification.

There is a stratification in the degree of overgrowth and production of hydrogen among the various diagnostic categories. For example, during the double blind study in the treatment of SIBO in fibromyalgia (Example 4), it was noted that the level of hydrogen production during the LBHT was much higher in this group of subjects as compared to those in subjects in the IBS incidence study described in Example 3. Given that the bacterial load is related to the level of hydrogen production, this implies that the degree of overgrowth is higher in patients with fibromyalgia compared to subjects with IBS.

The stratification of breath hydrogen levels with respect to diagnostic categories is as follows: IBS/Crohn's Disease (40-70 ppm of hydrogen); CFS (50-100 ppm of hydrogen); and FM (100-250 ppm of hydrogen).

Example 8: Intestinal Dysmotility Associated with IBS and FM.

Clinical experience showed that SIBO tends to recur after anti-biotic treatment within about 2 months. To demonstrate that a lack of phase III interdigestive motility is responsible for SIBO in subjects with IBS or fibromyalgia, antroduodenal manometry was conducted in human subjects diagnosed with IBS or FM.

Antroduodenal Manometry. Phase III interdigestive (fasting) motility was assessed in 15 human subjects. An antroduodenal manometry was performed by placing an 8-channel small bowel manometry catheter (each channel spaced 5 cm apart) into the small bowel using fluoroscopic guidance. After placement of the catheter, manometric recordings were made with an Arndorffer perfusion system with signals collected using Medtronic/Synectics Polygraf and associated Polygram software. Data were assessed for the characteristics of interdigestive motility.

IBS. Phase III interdigestive motility was assessed for a six-hour period in 15 human subjects having a suspected diagnosis of IBS, as defined by Rome Criteria, corroborated by concomitant SIBO. Of these 15 subjects, 13 (86%) had no detectable phase III interdigestive motility during the period of study. One subject (7%) had phase III interdigestive motility of short duration (<3 minutes), and one subject (7%) had normal phase III interdigestive motility.

Fibromyalgia. Phase III interdigestive motility was assessed in seven human subjects having a suspected diagnosis of fibromyalgia corroborated by the presence of SIBO. Of these seven subjects, six (86%) lacked detectable phase III interdigestive motility, and one subject (14%) had motility of less than normal peristaltic amplitude. The duration of study in the patients with fibromyalgia averaged 216 ± 45 minutes in the fasting state.

Example 9a: Treatment of SIBO-related IBS with a Prokinetic Agent.

Erythromycin, as a motilin agonist, can induce phase III of interdigestive motility. (E.g., M.J. Clark *et al.*, *Erythromycin derivatives ABT229 and GM 611 act on motilin receptors in the rabbit duodenum*, Clin. Exp. Pharmacol. Physiol. 26(3):242-45 [1999]).

Therefore, two subjects with recurrent IBS symptoms received prokinetic treatment with erythromycin.

The two subjects were a 55-year-old female and a 43-year-old female, both diagnosed with IBS. SIBO was detected in these subjects by LBHT. Antibiotic treatment of the SIBO resulted in greater than 90% improvement in symptoms. However, IBS symptoms recurred three to four weeks later, concurrent with a return of the SIBO condition. Subsequent courses of antibiotic treatment resulted in a similar pattern of improvement followed by a rapid recurrence of IBS symptoms in both subjects. Antroduodenal manometry was performed, demonstrating a lack of phase III of interdigestive motility, and erythromycin (50 mg daily) was prescribed to the subjects. The two subjects subsequently remained free of IBS symptoms and SIBO for at least 18 months and six months, respectively.

These results demonstrate the effectiveness of prokinetic treatment with erythromycin in preventing the recurrence of SIBO and IBS symptoms in subjects diagnosed with IBS.

15 Example 9b: Treatment of SIBO-related IBS with a Supplemental Pancreatic Enzyme.

Supplementing food with pancreatic enzymes facilitates more efficient absorption and digestion of food nutrients, thus allowing ingested food nutrients to be absorbed higher up in the small intestine than otherwise. This leads to a relative deprivation of nutrients to the bacteria involved in the SIBO condition. An example of this treatment modality occurred in the case of a 19-year-old male who had longstanding history of altered bowel habits, bloating, gas, distension and significant urge to evacuate. All of these symptoms were consistent with irritable bowel syndrome (IBS). The patient was diagnosed as having SIBO based on the results of LBHT. Subsequent to treatment with antibiotics, the patient had significant improvement in his symptoms. However, his SIBO condition became difficult to manage due to antibiotic resistance. An alternative treatment regimen was prescribed, which involved the addition of a pancreatic enzyme to the patient's food (10,000 Units human pancrease in capsules ingested immediately before each meal). With this therapy, the patient reported that his gastrointestinal complaints have improved by approximately 30-40%, corresponding to partial eradication of his SIBO condition. Treatment was continued for at least eight months with a continuation of the improvement in symptoms during that period.

Example 9c: Excessive Methane Production in Subjects with Small Intestinal Bacterial

Overgrowth is Associated with Less Diarrhea.

Bacterial metabolism is the major mechanism for the removal of hydrogen that is produced during fermentation reactions of intestinal bacteria. Specifically, hydrogen is consumed in the production of methane and in the reduction of sulfates to sulfides, with the 2 pathways being mutually exclusive. Since intestinal sulfides are known to be damaging to intestinal epithelium, it was hypothesized that diarrhea may be a less prevalent symptom among patients with small intestinal bacterial overgrowth (SIBO) who test positive for methane (no damaging sulfides produced).

Subjects referred to the Cedars-Sinai GI Motility Program for LBHT were entered into a database. Subjects were asked to rate symptoms of bloating, diarrhea, constipation, abdominal pain, mucous in stool, incomplete evacuation, straining and urgency, on visual analogue scales (0-5, with 0 representing no symptoms). An ANOVA was used to compare symptom scores between subjects producing no measured gases (only sulfide producing bacteria), H₂ only, H₂ and CH₄, and CH₄ only, on the LBHT.

Of the 771 subjects in the database, 48 were excluded because they demonstrated rapid transit on the LBHT. Of the 723 subjects remaining, 514 were positive for SIBO and 43 were considered non-methane, non-hydrogen producers. Among the 514 who had SIBO, 435 (85%) produced H₂ only, 68 (13%) produced both H₂ and CH₄, and 11 (2%) produced CH₄ only. The severity of diarrhea was highest in the non- H₂, non CH₄ and H₂ only group with less in the H₂ and CH₄ group, and CH₄ only group. There was a significant difference between the three groups for diarrhea ($p < 0.00001$ after Bonferroni correction). Urgency demonstrated the same trend, but was not significantly different. All other symptoms were no different. The severity of diarrheal symptoms is less in SIBO patients who excrete methane (Figure 7). In the non-methane-producers, greater severity of diarrheal symptoms likely reflects the reduction of sulfates to sulfides as the alternate pathway for the removal of hydrogen.

Example 10: Treatment of SIBO-related hyperalgesia

An adult male subject with a suspected diagnosis of IBS was found to have SIBO, as detected by LBHT. Anorectal manometry revealed rectal hypersensitivity in this subject. After eradication of his SIBO condition with antibiotic treatment, a repeat anorectal manometry showed that his rectal hyperalgesia had resolved.

Two adult female subjects with IBS required additional pharmacologic manipulations

to treat their SIBO-related hyperalgesia. In the first case, SIBO was eradicated by antibiotic treatment. However, the subject complained of persistent feelings of rectal distension, consistent with residual hyperalgesia related to SIBO. The subject was then administered Colpermin (peppermint oil) capsules and Elavil (5 mg taken at night) that alleviated her SIBO-related hyperalgesic symptoms, presumably by reducing intestinal wall tension and decreasing mechanoreceptor activation.

The second female subject with a diagnosis of IBS was also found to have SIBO, as detected by LBHT. Her SIBO was eradicated by a combined treatment with antibiotic, intestinal lavage with Go-Lytely, and cisapride (10 mg tid) to increase her abnormally low phase III interdigestive motility. After eradication of SIBO, this subject similarly complained of persistent SIBO-related hyperalgesic symptoms of the bowel. Administration of Colpermin (peppermint oil) then successfully alleviated the hyperalgesia, presumably by reducing the mechanoreceptor feedback for rectal distension.

Example 11: Treatment of SIBO using predigested nutritional formula

Based on the hypothesis that SIBO is promoted by nutritional components in food arriving at the distal gut, where they are used for carbon and energy by bacterial populations responsible for the SIBO condition, ten patients (8 female; 2 male; age range 17-64 years old; none having had a bowel resection) each diagnosed with IBS in accordance with the Rome Criteria, and each having SIBO as determined by LBHT, were treated with a total enteral nutrition (TEN) formula, which is absorbed in the proximal gut (Vivonex® T.E.N.; Sandoz Nutrition, Minneapolis, MN). Vivonex is a glutamine-enriched total enteral nutrition product, containing protein as free amino acids in a 56:44 essential to nonessential amino acid ratio, and inter alia, carbohydrate as maltodextrin and modified starch, safflower oil, and all essential vitamins and minerals. Vivonex is available as a powder for aqueous reconstitution (2.84 oz. packet; 1 packet mixed with 250 mL H₂O delivers 300 mL of formula). Each patient was administered an amount of reconstituted Vivonex to meet daily caloric needs according to the manufacturer's instructions, based on the each patient's weight, height and other relevant factors. The patient's were allowed no other nutritional intake, but water was allowed freely. After 14 days of the TEN regimen, each patient resumed his or her normal diet.

Figure 8 shows a representative result. In Figure 8A (pre-treatment), SIBO was initially detected by LBHT. After 14 days of the TEN regimen, follow-up LBHT shows that

SIBO had been at least partially eradicated (Figure 8B). Eradication was complete in eight of the patients with a greater than 80% improvement in IBS symptoms. Two of the patients had only partial eradication of SIBO with <20% improvement in IBS symptoms. The eradication of SIBO was maintained for up to two months after the TEN regimen was discontinued and normal nutrition had been resumed.

Example 12: Use of Active Lipids to Treat SIBO-related conditions

10 Oleate and Oleic Acid Slow Upper Gut Transit and Reduce Diarrhea in Patients with Rapid Upper Gut Transit and Diarrhea

Rapid transit through the upper gut can result in diarrhea, maldigestion and absorption, and weight loss; and pharmacologic treatment with opiates or anticholinergics often is required. It was tested whether fatty acids could be used to slow upper gut transit and reduce diarrhea in patients with rapid transit and diarrhea.

15 In a preliminary study, five patients with persistent diarrhea for 3 to 22 months, (one each due to vagal denervation, ileal resection for Crohn's disease, and vagotomy and antrectomy, and two due to idiopathic causes) were studied. Each patient demonstrated rapid upper gut transit on routine lactulose breath hydrogen testing (or variations thereof measuring labelled carbon dioxide)(Cammack *et al. Gut* 23:957-961 [1982]). This test relies on the metabolism of certain carbohydrate materials (e.g. lactulose) by the microbial flora within the caecum. By generating gas which can be detected in the expired air, it is possible to make some estimation about the initial arrival of the administered material within the colon.

25 Each patient received orally in random order, 0, 1.6 or 3.2 g of sodium oleate in 25 mL Ensure (Ross), followed by 100 mL water. Thirty minutes after each dose of oleate, patients received 10 g lactulose orally, followed by 25 mL water. Breath samples were collected in commercially available breath testing bags (Quintron, Menomonee Falls, WI) every 10-15 minutes, and the hydrogen content of the samples was measured using a breath analyzer (Microlyzer Model 12, Quintron Instruments, Menomonee Falls, WI), calibrated against gas samples of known hydrogen concentration. With a syringe, a 40-mL sample of the expired breath was withdrawn from the collection bag and analyzed immediately for hydrogen concentration (ppm). The hydrogen concentration value from each

sample was plotted against time. Upper gut transit time was defined as the time in minutes from ingestion of lactulose (t_0) until a rise of H_2 of >10 ppm. Data were further analyzed using 1-way repeated measures analysis of variance (ANOVA)(See Table 4).

5 Table 4. Effect of oleate on upper gut transit time (mean \pm SE).

| Oleate (g) | 0 | 1.6 | 3.2 |
|--------------------|--------------|----------------|----------------|
| Transit time (min) | 46 \pm 8.6 | 116 \pm 11.1 | 140 \pm 11.5 |

10 Upper gut transit was significantly prolonged by oleate in a dose-dependent fashion ($p < 0.005$, significant trend). During prolonged ingestion of oleate 15-30 minutes prior to meals, all patients reported reduced diarrhea. The patient with Crohn's disease reported complete resolution of chronic abdominal pain as well as post prandial bloating and nausea, and gained 22 lbs. In addition, the patient with vagotomy and antrectomy reported
15 resolution of postprandial dumping syndrome (flushing, nausea, light-headedness).

The effect of an active lipid on transit time was determined in 8 normal human subjects (1 male and 7 females with a mean age of 35 ± 2.6 years [SE]) and 45 patients (20 males and 25 females with a mean age of 49.1 ± 2.5 [SE], age range from 18 to 90 years) with chronic diarrhea (i.e., continuous diarrhea for more than two months) associated with
20 a wide variety of diagnoses and conditions (e.g., Crohn's disease; irritable bowel syndrome; short bowel syndrome; Indiana pouch; AIDS; ulcerative colitis; vagotomy; antrectomy; ileostomy; partial and complete colectomy; colon cancer; diabetes mellitus type 1; pancreatic insufficiency; radiation enteropathy; esophagectomy/gastric pull-up; total and subtotal gastrectomy; gastorjejunostomy), made by referring gastroenterologists. The method was
25 the same as described above, except oleic acid (Penta Manufacturing, Livingston, NJ) replaced sodium oleate in 50 mL of Ensure emulsion. All subjects refrained from taking antibiotics for at least two weeks before each testing date and during stool measurement periods. Patients were also instructed to refrain from anti-diarrheal drugs, laxatives, somatostatin analogues or anticholinergics for at least 48 hours before each test. In both the
30 normal and patient groups, there was a significant slowing of upper gut transit time in response to oleic acid, as summarized in Table 5 below ($p < 0.001$).

Table 5. Effect of Oleic Acid on upper gut transit time.

| <u>Transit time (min) (mean \pm SE)</u> | | | |
|--|------------------|----------------|----------------|
| <u>Oleic Acid (g)</u> | <u>0</u> | <u>1.6</u> | <u>3.2</u> |
| Normal | 105.2 \pm 12.1 | 116 \pm 11.1 | 140 \pm 11.5 |
| Patients | 29.3 \pm 2.8 | 57.2 \pm 4.5 | 83.3 \pm 5.2 |

5

Continuing oleic acid treatment at home was offered to responders (i.e., patients who experienced a greater than 100 % increase in baseline transit time with 3.2 g oleic acid). Of the 36 responders out of the original 45 patients, 18 provided records of stool volume and frequency on- and off- treatment for comparison. The inconvenient and unappealing nature of stool collection and measurement were the primary reasons reported by responders who chose not to participate in stool collection. After completing a set of three preliminary breath hydrogen tests, each participating responder was asked to refrain from taking oleic acid for two days in order to measure off-treatment stool output for a 24-hour period. Patients were issued a stool pattern record form and a stool collection container with graduated volume markings to record the frequency and volume of bowel movements. After two days without oleic acid, each patient took 3.2 g of oleic acid mixed with 25 mL of Ensure emulsion three times a day, 30 minutes before breakfast, lunch and dinner. After taking oleic acid for two days, patients recorded stool output for another 24-hour period. With this oleic acid emulsion treatment, stool frequency decreased from 6.9 ± 0.8 to 5.4 ± 0.9 bowel movements per 24-hour period ($p < 0.05$), and stool volume decreased from 1829.0 ± 368.6 to 1322.5 ± 256.9 per 24-hour period ($p < 0.05$). A slight and transient burning sensation in the mouth or throat was the only adverse effect reported by any patient taking the oleic acid treatment.

These experiments demonstrate that active lipids, such as oleate and oleic acid, are effective in slowing upper gut transit in a dose-dependent manner, thus enabling longer residence time for food in the upper gut and a concomitant greater nutrient absorption there.

Fat in Distal Gut Inhibits Intestinal Transit More Potently Than Fat in Proximal Gut.

30

In 4 dogs equipped with duodenal (10 cm from pylorus) and mid-gut (160 cm from pylorus) fistulas, as described hereinbelow (Example 14), intestinal transit was compared across an isolated 150 cm test segment (between fistulas) while 0, 15, 30 or 60 mM oleate was delivered into either the proximal or distal segment of the gut as a solution of mixed micelles in pH 7.0 phosphate buffer at 2 mL/min for 90 minutes. The segment of gut

not receiving oleate was perfused with phosphate buffer, pH 7.0, at 2 mL/min. 60 minutes after the start of the perfusion, ~20 μ Ci of ^{99m}Tc -DTPA (diethylenetriaminepentaacetic acid) was delivered as a bolus into the test segment. Intestinal transit was then measured by counting the radioactivity of 1 ml samples collected every 5 minutes from the diverted output of the mid-gut fistula.

Intestinal transit was calculated by determining the area under the curve (AUC) of the cumulative percent recovery of the radioactive marker. The square root values of the AUC (Sqrt AUC), where 0 = no recovery by 30 minutes and 47.4 = theoretical, instantaneous complete recovery by time 0, were compared across region of fat exposure and oleate dose using 2-way repeated measures ANOVA (see Table 6 below).

Table 6. Effect of Oleate and oleic acid on intestinal transit.

| Region of fat exposure | Oleate dose (mM) (mean \pm SE) | | |
|------------------------|----------------------------------|-----------------|----------------|
| | 15 | 30 | 60 |
| Proximal 1/2 of gut | 41.6 \pm 1.4 | 40.6 \pm 10.2 | 34.4 \pm 3.0 |
| Distal 1/2 of gut | 25.6 \pm 1.4 | 18.9 \pm 1.5 | 7.0 \pm 3.8 |

Control: buffer into both proximal and distal 1/2 of gut = 41.4 \pm 4.6.

These experiments demonstrate that intestinal transit is slower when fat is exposed in the distal 1/2 of gut (region effect $p < .01$). These experiments also demonstrate that oleate is effective to inhibit intestinal transit in a dose-dependent fashion (dose effect, $p < .05$); and that dose dependent inhibition of intestinal transit by oleate depends on the region of exposure (interaction between region and dose, $p < 0.01$).

Case Study Showing Successful Treatment of Diarrhea-Predominant Irritable Bowel Syndrome With Oleic Acid. The patient was a 39-year old male with a history of adolescent-onset, persistent diarrhea. After a routine gastrointestinal work-up failed to provide an explanation for his symptoms, he was given the diagnosis of diarrhea-predominant irritable bowel syndrome. He presented with complaints of excessive gas, postprandial bloating, diarrhea and urgency, and 3 to 7 liquid bowel movements per day. His upper gut transit times were (min) 30 (0 g oleic acid), 117 (1.6 g oleic acid) and 101 (3.2 g oleic acid). With continuing oleic acid treatment as described above, he reported his bowel

frequency reduced to a single, solid bowel movement per day. He also reported complete relief from the symptoms of gaseousness, bloating and rectal urgency.

Relatively rapid basal upper gut transit in Patients with Inflammatory Bowel Disease (IBD).

5 The mean upper gut transit time for IBD patients (n=18) at 0 grams of oleic acid was 79.1 ± 11.0 min., compared to 118.7 ± 9.8 min for normal subjects (n = 5)(p = 0.04, t-test).

Active lipid increases upper gut transit time. The mean transit time for normal subjects (n=5) at 0 grams of oleic acid was 118.7 ± 9.8 min, at 4 grams of Oleic acid was 136.0 ± 15.4 min. (P <0.05, t-test). The mean AUC for normal subjects at 0 grams of oleic acid was 1438.9 ± 208.5 ; at 4 grams of oleic acid it was 1873.3 ± 330.5 (p < 0.05, t-test). The mean transit time for IBD patients (n=18) at 0 grams of oleic acid was 79.1 ± 11.0 min; at 4 grams of oleic acid it was 114.6 ± 16.0 min. (p < 0.05, t-test). The mean AUC for IBD patients at 0 grams of oleic acid was 687.3 ± 98.2 ; at 4 grams of oleic acid it was 1244.9 ± 250.4 . (p

15 < 0.05, t-test).

These data show that oleic acid slowed gut transit time and thus substantially increased the opportunity for absorption of food nutrients in the upper gut region in both normal and IBD groups. Thus, the in individuals having SIBO a condition, treatment in accordance with the method of deprives the bacteria of much of the nutrient supply required

20 for growth.

Example 13: Eradication of SIBO in subjects with irritable bowel syndrome lowers their serum levels of 5-HT.

Previous studies have shown that patients with irritable bowel syndrome (IBS) have

25 elevated plasma 5-hydroxytryptamine (5-HT) levels. Since it was shown hereinabove that IBS is associated with small intestinal bacterial overgrowth (SIBO) and symptoms of IBS are reduced by antibiotic eradication of SIBO, the hypothesis was tested that eradication of SIBO will reduce plasma 5-HT levels in IBS patients to provide further evidence of the relationship between IBS and SIBO.

30 The plasma 5-HT levels of 7 human subjects diagnosed with IBS were compared before and after successful eradication of SIBO, as part of a double blind placebo controlled trial. A lactulose breath hydrogen test (LBHT) was performed to diagnose SIBO at baseline and when eradication was achieved. Fasting blood samples were taken at baseline and on the

day that eradication of SIBO was confirmed. The plasma 5-HT level (ng/mL) was determined in each sample by ELISA (Kit-Research Diagnostics Inc., Flanders, NJ). A paired t-test was performed to compare 5HT levels (mean \pm SE) before and after eradication of SIBO.

- 5 The results indicated that the amount of plasma 5-HT was reduced from 0.7 ± 0.4 ng/mL before eradication to 0.5 ± 0.5 ng/mL after eradication of SIBO in the subjects ($p < 0.05$). Thus, eradication of SIBO in IBS subjects decreases fasting plasma 5-HT levels, which provides further evidence for the relationship between IBS and SIBO.

10 Example 14: Neural Regulation of the Rate of Upper Gastrointestinal Transit

The experiments described below are based on a previously described chronic multi-fistulated dog model, employing surgically fistulated male or female mongrel dogs weighing about 25 kg each. (Lin, H.C. et al., *Inhibition of gastric emptying by glucose depends on*
15 *length of intestine exposed to nutrient*, Am. J. Physiol. 256:G404-G411 [1989]). The small intestines of the dogs were each about 300 cm long from the pylorus to the ileal-cecal valve. The duodenal fistula was situated 15 cm from the pylorus; the mid-gut fistula was situated 160 cm from the pylorus. Occluding Foley catheters (balloon catheters that are inflated to produce a water-tight seal with the luminal surface) were placed into the distal limb of a
20 duodenal fistula and a mid-gut fistula, fat or other test agents were administered lumenally to the thus compartmentalized proximal section of the gut, i.e., between the fistulas, or to the compartmentalized distal section of the gut, i.e., beyond the mid-gut fistula. Perfusate was pumped into a test section through the catheter at a rate of 2mL/minute. Test agents were administered along with buffer perfusate, but some test agents were administered
25 intravenously, where specifically noted.

Intestinal transit measurements were made by tracking the movement of a liquid marker across the approximately 150 cm intestinal test segment by delivering about 20 μ Ci 99m Tc chelated to diethyltriamine pentaacetic acid (DTPA)(Cunningham, K.M. et al., *Use of technetium-99m (V)thiocyanate to measure gastric emptying of fat*, J. Nucl. Med. 32:878-881
30 [1991]) as a bolus into the test segment after 60 minutes of a 90-minute perfusion. The output from the mid-gut fistula was collected every 5 min thereafter for 30 minutes, which period is illustrated in Figures 9-23. Using a matched dose of 99m Tc to represent the original radioactivity (Johansson, C., *Studies of gastrointestinal interactions*, Scand. J. Gastroenterol. 9(Suppl 28):1-60 [1974]; Zierler, K., *A simplified explanation of the theory of indicator*

dilution for measurement of fluid flow and volume and other distributive phenomena, Bull. John Hopkins 103:199-217 [1958]), the radioactivity delivered into the animal as well as the radioactivity of the recovered fistula output were all measured using a gamma well counter.

After correcting all counts to time zero, intestinal transit was calculated as the cumulative percent recovery of the delivered ^{99m}Tc -DTPA. This method has been well validated over the years and appreciated for its advantage of minimal inadvertent marker loss. To demonstrate this point, we perfused phosphate buffer, pH 7.0, through the proximal gut and followed the cumulative recovery of this marker (% recovery) over time ($n=1$). There was a very high level of marker recovery, with 90% of the marker recovered by 30 minutes and 98% of the marker recovered by 45 minutes.

(1) Slowing of intestinal transit by PYY depends on ondansetron-sensitive 5-HT-mediated pathway. Peptide YY (PYY) slows transit and is a signal for luminal fat (Lin, H.C. *et al.*, *Fat-induced ileal brake in the dog depends on peptide YY*, Gastroenterol. 110(5):1491-95 [1996b]; Lin, H.C. *et al.*, *Slowing of intestinal transit by fat in proximal gut depends on peptide YY*, Neurogastroenterol. Motility 10:82 [1998]). Since serotonin (5-HT) can also be a signal for fat (Brown, N.J. *et al.*, *The effect of a 5HT3 antagonist on the ileal brake mechanism in the rat*, J. Pharmacol. 43:517-19 [1991]; Brown, N.J. *et al.* [1993]), the hypothesis was tested that the slowing of transit by PYY can depend on a 5-HT-mediated pathway by comparing the rate of marker transit during the administration of PYY in the presence or absence of ondansetron (Ond; a 5-HT receptor antagonist) in the proximal versus distal gut ($n = 2$ for each treatment).

Normal saline (0.15 M NaCl) or PYY (0.8 $\mu\text{g/kg/h}$) was administered intravenously over a 90 minute period, while phosphate buffer, pH 7.0, was perfused into the lumen of the proximal gut through the duodenal fistula at a rate of 2 mL/min for the 90 minutes and was recovered from the output of the mid-gut fistula. The results are summarized in Figure 9.

Transit was slowed by intravenous PYY, with recovery of the marker decreased from $75.1 \pm 3.6\%$ (control: IV normal saline [NS] + luminal normal saline, i.e., NS-NS in Figure 9) to $17.1 \pm 11.0\%$ (IV PYY + luminal normal saline, i.e., PYY-NS in Figure 9). This effect was abolished by adding the specific 5-HT₃ receptor antagonist ondansetron (0.7 mg/kg/h) to the buffer introduced into the proximal gut so that recovery increased to $78.3 \pm 4.8\%$ (IV PYY + luminal Ond proximal, i.e., PYY-Ond in prox in Figure 9) but not by ondansetron in the distal gut, which decreased recovery to $12.9 \pm 12.9\%$ (IV PYY + Ond in Distal, i.e.,

PYY-Ond in Dist). These results imply that slowing of transit by PYY depended on a 5-HT-mediated pathway located in the segment of the small intestine where transit was measured.

(2) The fat induced jejunal brake depends on an ondansetron-sensitive serotonin (5-HT)-mediated pathway. The hypothesis was tested that slowing of transit by fat depends on a serotonergic pathway by comparing intestinal transit during perfusion with buffer or oleate in the presence or absence of ondansetron, a 5-HT₃ receptor antagonist, in the proximal gut (n=3 each treatment). Buffer or 60 mM oleate was perfused through the duodenal fistula into the lumen of the proximal gut for a 90-minute period, in the manner described in Example 14(1), along with a bolus of normal saline ± ondansetron (0.7 mg/kg) at the start of transit measurement. The rate of intestinal transit was slowed by the presence of oleate (p<0.05) in an ondansetron-sensitive manner. (p<0.05). The results are summarized in Figure 10.

Specifically, ondansetron increased recovery of marker in the perfusate from $41.6 \pm 4.6\%$ (mean ± SE) (luminal oleate + luminal normal saline, i.e., Oleate-NS in Figure 10) to $73.7 \pm 10.6\%$ (luminal oleate + luminal ondansetron, i.e., Oleate-Ond in Figure 10) during oleate perfusion but decreased recovery from $96.0 \pm 4.0\%$ (luminal phosphate buffer + luminal normal saline, i.e., Buffer-NS in Figure 10) to $57.9 \pm 15.9\%$ (luminal buffer + luminal ondansetron, i.e., Buffer-Ond in Figure 10) during buffer perfusion. These results imply that slowing of intestinal transit by the fat-induced jejunal brake and the acceleration of intestinal transit by buffer distension both depended on an ondansetron-sensitive 5-HT₃-mediated pathway.

(3) The fat-induced ileal brake depends on an ondansetron-sensitive, efferent serotonin (5-HT)-mediated pathway. The fistulated dog model allows for the ileal brake (oleate in distal gut, buffer in proximal gut) to be separated into the afferent (distal) vs. efferent (proximal) limb of the response. Since 5-HT₃ receptors are found on extrinsic primary sensory neurons (afferent limb) and on intrinsic 5-HT neurons of the myenteric plexus (5-HT interneuron)(efferent limb), the identification of the location of the 5-HT₃ pathway (afferent vs. efferent limb) can localize the serotonergic pathway responsible for the slowing of transit by fat in the distal gut (ileal brake). Using occluding Foley catheters, the small intestine was compartmentalized into the proximal gut and the distal gut as described hereinabove. Intestinal transit was measured across the proximal gut (between fistulas) as described hereinabove. By perfusing buffer through the proximal gut while fat was perfused through

the distal gut to trigger the fat-induced ileal brake, the distal gut represented the afferent limb of the response and the proximal gut represented the efferent limb of the response. To test for the location of the serotonergic pathway, 5-HT₃ receptor antagonist ondansetron was then mixed with the appropriate perfusate and administered into either the proximal or distal gut.

5 Control = buffer in proximal and distal gut. Four dogs were tested.

Delivering ondansetron lumenally into either the proximal or distal gut, intestinal transit was slowed by the ileal brake ($76.3 \pm 3.1\%$ [Control in Figure 11] vs. $22.9 \pm 3.8\%$ [Ileal Brake in Figure 11]; $p < 0.005$). But the ileal brake was abolished by ondansetron delivered to the proximal gut ($68.5 \pm 2.7\%$; Ond in Prox in Figure 11; $n = 4$) but not distal
10 gut ($22.8 \pm 2.6\%$; Ond in Dist in Figure 11; $n = 4$).

Since ondansetron delivered with the fat in the distal gut had no effect, but ondansetron delivered with the buffer in the proximal gut abolished the ileal brake, the slowing of intestinal transit by fat in the distal gut depended on an ondansetron-sensitive, serotonergic pathway located on the efferent rather than afferent limb of the response. And
15 since ondansetron abolished the jejunal brake in Example 14(2) when delivered with fat and abolished the ileal brake in Example 14(3) when delivered with buffer, this region-specific result cannot be explained by inactivation of drug by fat, differences in permeability or absorption.

20 (4) Ondansetron abolishes the fat-induced ileal brake in a dose-dependent manner. The fat-induced ileal brake was abolished by the 5-HT receptor antagonist ondansetron in a dose-dependent manner. Perfusion of buffer was through both the duodenal and mid-gut fistulas (2 mL/min over 90 minutes); the buffer administered to the mid-gut fistula contained buffered normal saline (pH = 7.0; Buffer Control in Figure 12) or 60 mM oleate to induce
25 the ileal brake response (Ileal Brake in Figure 12). During the ileal brake response, ondansetron was added at t_0 as a single bolus in the following doses (mg): 6.25; 12.5; and 25. Results are shown in Figure 12.

Oleate induced the ileal brake (24.1% marker recovery [Ileal brake in Figure 12] vs. 81.2% marker recovery for the Buffer Control). The ileal brake was abolished
30 by ondansetron delivered into the proximal gut in a dose-dependent manner (35.4% marker recovery at 6.25 mg ondansetron, 55.8% marker recovery at 12.5 mg ondansetron, and 77.6% marker recovery at 25 mg ondansetron).

(5) Fat in the distal gut causes the release of 5-HT from the proximal gut. To test the hypothesis that fat in the distal gut causes the release of 5-HT in the proximal gut, the amount of 5-HT collected from the output of the mid-gut fistula (proximal gut 5-HT) over a 90-minute period of buffer perfusion through both the duodenal and mid-gut fistulas (2 mL/min); buffer (control) or oleate (60 mM) was administered to the distal gut (n=1). The amount of 5-HT was determined using an ELISA kit specific for 5-HT (Sigma; Graham-Smith, D.G., *The carcinoid syndrome*, In: *Topics in Gastroenterology*, Truclove, S.C. and Lee, E. (eds.), Blackwells, London, p. 275 [1977]; Singh, S.M. *et al.*, *Concentrations of serotonin in plasma-- a test for appendicitis?*, Clin. Chem. 34:2572-2574 [1988]). The amount of 5-HT released by the proximal gut increased in response to fat in the distal gut from 100 µg in the control (buffer minus oleate) to 338 µg (buffer plus oleate to distal gut), showing that 5-HT is released in the proximal gut in response to fat in the distal gut. Thus, the release of 5-HT by the proximal gut can serve as a relayed signal for fat in the distal gut. The relayed release of 5-HT in the proximal gut in response to fat in the distal gut is consistent with Example 14(2), showing that slowing of intestinal transit by fat depends on an efferent 5-HT-mediated pathway to the proximal gut.

(6) Ondansetron abolishes the fat-induced ileal brake when administered lumenally but not intravenously. To confirm that the reversal of the slowing of transit by ondansetron was peripheral, i.e., enteric, rather than systemic, the effect of ondansetron was compared when delivered lumenally (through the duodenal fistula into the proximal gut) versus intravenously. Ondansetron was either delivered lumenally into the proximal gut (0.7 mg/kg/h; Ond in prox in Figure 13) or administered intravenously (0.15 mg/kg/1.5h; iv Ond in Figure 13) during fat-induced ileal brake (60 mM oleate input through the mid-gut fistula into the distal gut as described above). Two dogs were tested (n = 2).

Results are shown in Figure 13. Compared to the ileal brake ($20 \pm 1.8\%$ marker recovery), the marker recovery increased to $78 \pm 2.4\%$ with luminal ondansetron ($p < 0.005$). Intravenous ondansetron had no substantial effect on the ileal brake ($13 \pm 2.0\%$ marker recovery). These results imply that the 5-HT₃ receptor antagonist worked enterically rather than systemically.

(7) The slowing of intestinal transit by distal gut 5-HT depends on an ondansetron-sensitive 5-HT₃-mediated pathway in the proximal gut (efferent) and distal gut (afferent).

To test the hypothesis that luminal 5-HT may slow intestinal transit via 5-HT₃ receptors similar to fat, 0.7 mg/kg ondansetron, a 5-HT₃ receptor antagonist or buffered saline (pH 7.0) was delivered into either the proximal or distal gut as a bolus at the start of the transit measurement. Four dogs were tested.

5 Results are shown in Figure 14. The slowing of intestinal transit by 5-HT (0.1 mg/kg/h) administered to the distal gut ($35.2 \pm 2.2\%$ marker recovery) (vs. $76.1 \pm 4.7\%$ marker recovery for buffer control) was abolished by ondansetron added to the proximal or distal gut as shown by % marker recovery of $73.8 \pm 9.5\%$ (Ond-Prox in Figure 14) vs. $79.5 \pm 2.4\%$ (Ond-Dist in Figure 14), respectively ($p < 0.001$).

10 This shows that in the conscious whole animal, the slowing of intestinal transit by luminal 5-HT depended on an ondansetron-sensitive serotonergic pathway located on both the afferent and efferent limb of the intestino-intestinal reflex. (See also, Brown, N.J. *et al.*, *Granisetron and ondansetron: effects on the ileal brake mechanism in the rat*, J. Pharm. Pharmacol. 45(6):521-24 [1993]). In contrast, the slowing of intestinal transit by distal gut
15 fat (Example 14[3]) depended on a 5-HT₃ pathway localized specifically on the efferent limb to suggest that 5-HT is not the stimulus for the afferent limb of the fat-induced ileal brake, but rather involves a signal other than 5-HT, such as PYY. However, 5-HT is the stimulus for the afferent limb of the slowing of intestinal transit by 5-HT in the distal gut.

20 (8a) 5-HT in the distal gut slows intestinal transit in a dose-dependent manner. In a preliminary experiment, intestinal transit during buffer perfusion of both the proximal and distal guts (81.2% recovery) was slowed by 5-HT in distal gut so that marker recovery decreased to 73.8% at 2 mg 5-HT (0.033 mg 5-HT/kg/h), 53.1 % at 3 mg (0.05 mg 5-HT/kg/h) and 11.6% at 4 mg (0.066 mg 5-HT/kg/h) dose over a 90 minute period ($n = 1$).

25 The dose-dependent effect of 5-HT in slowing intestinal transit was confirmed in an additional experiment. The cumulative % recovery of the radioactive marker was reduced in a dose-dependent fashion as the 5-HT perfusion increased from 0 to 0.1 mg/kg/h to suggest that intestinal transit is slowed by luminal 5-HT. However, the speed of transit was markedly accelerated when the 5-HT dose was increased to 0.3 mg/kg/h.

30 (Table 7).

Table 7: Effect of 5-HT delivered to distal gut on intestinal transit time (min) in multi-fistulated dogs ($n = 2$ dogs).

5-HT dose (mg/kg/h x 90 min)

| | 0 | 0.033 | 0.05 | 0.066 | 0.1 | 0.3 |
|---|------------|------------|------------|------------|------------|------------|
| 5 | 68.5 ± 1.0 | 69.6 ± 4.2 | 33.5 ± 1.5 | 15.2 ± 0.5 | 16.1 ± 4.9 | 73.8 ± 0.6 |

(8b) Luminal 5-HT, delivered to the proximal gut, slows intestinal transit in a dose-dependent fashion in the conscious whole animal model. In in-vitro models, luminal 5-HT applied to an isolated bowel loop accelerated transit by triggering the peristaltic reflex. In contrast, in the conscious whole animal model applied herein (with extrinsic nerves intact), 5-HT applied lumenally slowed intestinal transit (Example 14[8a] above). In further experiments, 5-HT was delivered at a rate of 0, 0.033, 0.066, 0.05 and 0.1 mg/kg/h into the proximal gut. Four dogs were tested.

Results are shown in Figure 15. Intestinal transit was significantly slowed by 5-HT in the proximal gut in a dose-dependent fashion ($p < 0.00001$). Marker recovery during buffer perfusion was $75.0 \pm 4.4\%$ while at the dose of 0.066 mg/kg/h marker recovery was reduced to $16.9 \pm 3.7\%$, and was not significantly different from the dose of 0.1 mg/kg/h. At the intermediate dose of 0.05 mg/kg/h, marker recovery was $33.2 \pm 14.0\%$; buffer vs 0.05 mg/kg/h; $p < 0.005$) and at the lowest dose of 0.033 mg/kg/h, marker recovery was not significantly different from the buffer control.

(8c) Slowing of Intestinal Transit by 5-HT is not dependent on volume of the output of the midgut fistula. 5-HT stimulates small bowel and colonic secretion. We have observed a slowing effect of 5-HT on intestinal transit (Example 14[8a-b]). As a control, to determine whether intestinal transit correlates with volume of the output of the midgut fistula. Varying doses of 5-HT (0, 0.033, 0.1, 0.3 mg/kg/h) were perfused into the proximal gut, ^{99m}Tc was delivered into the test segment as a bolus for transit measurement. The volume of the output of the midgut fistula was collected during the last 30 minutes of the 90 min perfusion experiment ($n=21$). Transit was plotted against output volume. There was no correlation between transit during 5-HT treatment and the volume of the output of the midgut fistula (data not shown).

Therefore, the observed transit effect of 5-HT cannot be explained solely on the basis of volume effect related to 5-HT induced intestinal secretion. The observed transit effect of 5-HT must depend on transit-specific regulation.

Together, the results in Example 14(8) and show that, contrary to the effect of 5-HT in an in-vitro model, lumenally administered 5-HT slows intestinal transit in a dose-dependent fashion in the conscious whole animal model, which implies that the slowing of intestinal transit depends on extrinsic nerves.

(9a) 5-HT in the distal gut causes release of 5-HT in the proximal gut. To test the hypothesis that 5-HT in the distal gut causes the release of 5-HT in the proximal gut, the amount of 5-HT collected from the output at the mid-gut fistula (Proximal gut 5-HT) over a 90-minute period of buffer perfusion through both the duodenal and mid-gut fistulas (2 mL/min each) was compared in the presence or absence of 5-HT (0.05 mg/kg/h) administered to the distal gut (n=1). 5-HT concentration was determined using an ELISA kit specific for 5-HT (Sigma). The amount of 5-HT released by the proximal gut increased from 156 μ g in the control (minus distal 5-HT) to 450 μ g (plus 5-HT to distal gut), implying that 5-HT is released by the proximal gut in response to 5-HT in the distal gut. Thus, the release of 5-HT by the proximal gut can serve as a relayed signal for distal gut 5-HT. This relayed release of 5-HT in the proximal gut explains the results of Example 14(6) showing that the slowing of intestinal transit by distal gut 5-HT was abolished by ondansetron in the proximal gut (efferent limb of response) as well as in the distal gut (afferent limb of response).

(9b) Fat in distal gut releases 5-HT from proximal gut. To test the hypothesis that the proximal gut releases 5-HT in response to lipid in the distal gut, we compared the amount of 5-HT in the output of the midgut fistula (i.e., proximal gut 5-HT) with buffered saline (control) or oleate in the distal gut. The amount of 5-HT collected over 90 min was measured using a 5-HT-specific ELISA test kit, as described herein above. Four dogs were tested.

The amount of proximal gut 5-HT increased from 82.7 ± 20.53 ng to 211.75 ± 35.44 ng ($p < 0.005$) when the distal gut perfusate was switched from buffer to oleate, implying that 5-HT is released from the proximal gut in response to fat in the distal gut, as a relayed signal for fat.

Fat is also a chemical trigger for the release of 5-HT, thus these results are consistent with the release of 5-HT via a long distance, intestino-intestinal communications, or reflex.

(9c) Luminal 5-HT slows intestinal transit via activation of the intestino-intestinal reflex. To confirm that 5-HT, delivered lumenally, slowed intestinal transit via the activation of an intestino-intestinal reflex, we compared intestinal transit across the proximal one-half of gut while 0 (pH 7.0 buffered saline control) or 0.1 mg/kg/h of 5-HT was delivered into either the proximal or distal one-half of the gut. Four dogs were tested.

Results are shown in Figure 16. Intestinal transit across the proximal gut was slowed by 5-HT in either the proximal or distal gut, demonstrated by the marker recovery decreasing from $85.0 \pm 7.3\%$ (Saline-Prox in Figure 16) ($p < 0.005$) to $20.1 \pm 4.5\%$ for proximal gut 5-HT (5-HT-Prox in Figure 16) and $76.1 \pm 1.3\%$ (Saline-Dist in Figure 16) to $35.2 \pm 2.3\%$ (5-HT-Dist in Figure 16) ($p < 0.005$) for distal gut 5-HT.

These results imply that the slowing of intestinal transit by 5-HT depends on a long-distance, region-to-region reflex, since 5-HT administered into the distal gut slowed intestinal transit through the physically separate proximal gut.

(10) Intravenous PYY causes release of 5-HT in the proximal gut. The amount of 5-HT released from the proximal gut in response to intravenous PYY or buffered saline (Control) during buffer perfusion (2 mL/min over 90 minutes) through both the duodenal and mid-gut fistulas was measured to test the hypothesis that intravenous PYY (0.8 mg/kg/h) causes the release of 5-HT in the proximal gut. 5-HT was measured as in Example 14(9) above. The amount of 5-HT released by the proximal gut increased from 140.1 μ g (Control) to 463.1 μ g in response to intravenous PYY.

This result was comparable with the response when 60 mM oleate was administered to the distal gut (buffer only to the proximal gut) during the perfusion without intravenous PYY (509.8 μ g of 5-HT; $n=1$), which implies that the release of 5-HT in the proximal gut stimulated by fat in the distal gut can be mediated by PYY.

(11) Slowing of intestinal transit by fat in the distal gut depends on an extrinsic adrenergic neural pathway. A distension-induced intestino-intestinal inhibitory neural reflex projects through the celiac prevertebral celiac ganglion via a cholinergic afferent and an adrenergic efferent (Szurszewski, J.H. and King, B.H., *Physiology of prevertebral ganglia in mammals with special reference to interior mesenteric ganglion*, In: *Handbook of Physiology: The Gastrointestinal System*, Schultz, S.G. et al. (eds.), American Physiological Society, distributed by Oxford University Press, pp. 519-592 [1989]). Intestinal transit was measured

during fat perfusion of the distal small intestine in the presence or absence of intravenous propranolol (50 $\mu\text{g/kg/h}$; $n = 2$ dogs), a β -adrenoceptor antagonist, to test the hypothesis that the slowing of intestinal transit by fat in the distal gut also depends on an adrenergic pathway. Perfusion of buffer was through both the duodenal and mid-gut fistulas (2 mL/min over 90 minutes); the buffer administered to the mid-gut fistula contained 60 mM oleate. The results are illustrated in Figure 17.

Intestinal transit was slowed by distal gut fat ($79.7 \pm 5.8\%$ marker recovery [Buffer Control in Figure 17] compared to $25.8 \pm 5.2\%$ recovery with fat perfusion into the distal gut [Oleate-NS in Figure 17]). Intravenous propranolol abolished this jejunal brake effect so that recovery increased to $72.1 \pm 4.7\%$ (oleate + propranolol, i.e., Oleate-Prop in Figure 17), implying that the slowing of transit by fat in the distal gut depends on a propranolol-sensitive, adrenergic pathway. This result supports the hypothesis that the response to fat involves an adrenergic efferent, such as the extrinsic nerves projecting through the prevertebral ganglia.

(12) Slowing of intestinal transit by PYY depends on an extrinsic adrenergic neural pathway.

Intestinal transit during buffer perfusion of the proximal and distal small intestine in the presence or absence of intravenous propranolol (50 $\mu\text{g/kg/h}$; $n = 2$) was measured, to test the hypothesis that the slowing of intestinal transit by PYY (a fat signal) also depends on an adrenergic pathway. Perfusion was through both fistulas as described in Example 14(11) except that oleate was not administered to the distal gut, and, instead, 30 μg PYY (0.8 mg/kg/h) was administered intravenously during the 90 minute perfusion period. The results are summarized in Figure 18.

Slowing of intestinal transit by PYY ($78.1 \pm 2.2\%$ marker recovery minus PYY [Buffer Control in Figure 18] vs. $11.8 \pm 5.4\%$ recovery with intravenous PYY [PYY-NS in Figure 18]) was abolished by intravenous propranolol. In the presence of propranolol, marker recovery increased to $66.3 \pm 3.1\%$ (PYY-Prop in Figure 18). This result, consistent with the results of Example 14(11), implies that the slowing of transit by PYY depends on a propranolol-sensitive, adrenergic pathway, which supports the hypothesis that the response to PYY involves an adrenergic efferent such as the extrinsic nerves projecting through the prevertebral ganglia.

(13) Slowing of intestinal transit by 5-HT in the distal gut depends on a propranolol-sensitive

extrinsic adrenergic neural pathway. Intestinal transit during buffer perfusion of the proximal and distal small intestine in the presence or absence of intravenous propranolol (50 µg/kg/h; n = 2) was measured, to test the hypothesis that the slowing of intestinal transit by 5-HT in the distal gut also depends on an adrenergic pathway. Buffer perfusion was through both
5 fistulas as described in Example 14(12) except that 5-HT (0.05 mg/kg/h) was administered to the distal gut during the 90 minute perfusion period. The results are summarized in Figure 19.

Slowing of intestinal transit by 5-HT ($83.3 \pm 3.3\%$ marker recovery minus 5-HT [Buffer Control in Figure 19] vs. $36.1 \pm 2.3\%$ recovery with administration of 5-HT to the
10 distal gut [5-HT-NS in Figure 19]) was abolished by intravenous propranolol. In the presence of propranolol, marker recovery increased to $77.7 \pm 7.6\%$ (5-HT-Prop in Figure 19).

This result implies that the slowing of transit by 5-HT depends on a propranolol-sensitive, extrinsic adrenergic pathway, perhaps similar to that responsible for the response to distal gut fat.

15 Enterochromaffin cells of the intestinal mucosa and myenteric 5-HT neurons are innervated by adrenergic nerves. (Gershon MD, Sherman DL., *Noradrenergic innervation of serotonergic neurons in the myenteric plexus*, J Comp Neurol. 1987 May 8;259(2):193-210 [1987]). To test the hypothesis that the slowing of intestinal transit by distal gut fat (ileal brake) and 5-HT depended on an adrenergic pathway, five dogs were equipped with duodenal
20 (10 cm from the pylorus) and midgut (160 cm from the pylorus) fistulas as described above.

Using occluding Foley catheters, the small intestine was compartmentalized into the proximal (between fistulas) and distal (beyond midgut fistula) one-half of gut. Buffer (pH 7.0) was perfused into the proximal gut while 60 mM oleate was perfused into the distal gut at 2 ml/min for 90 min. Intestinal transit across the proximal gut was compared during
25 intravenous administration of 50 µg/kg/h propranolol or saline. In addition, the effect was also determined of 5-HT administered at 0.1 mg/kg/h on intestinal transit with and without i.v. propranolol. Intestinal transit (mean \pm SE) was measured by ^{99m}Tc -DTPA marker recovery in the output of the midgut fistula during the last 30 min of the 90 min experiment.

The cumulative % marker recovered was compared using ANOVA and additional analyses
30 by paired t-test.

Results are shown in Table 8 below. Oleate ($p < 0.002$) and 5-HT ($p < 0.005$) perfused into the distal gut slowed transit through the proximal gut as compared to buffer control. The slowing of intestinal transit by distal gut fat or 5-HT was both abolished by iv

propranolol ($p < 0.01$). These results provide further evidence that the slowing of intestinal transit by distal gut fat or 5-HT depends on an adrenergic efferent nerve.

Table 8. Effect of 5-HT and propranolol on proximal intestinal transit.

| Perfusate | i.v. Agent | |
|---------------------|------------------|--------------------|
| | Saline (i.v.) | Propranolol (i.v.) |
| Buffer Control | 70.11 \pm 6.51 | - |
| Oleate (Peal brake) | 26.62 \pm 5.36 | 66.42 \pm 8.26 |
| 5-HT distal gut | 28.27 \pm 5.03 | 63.85 \pm 8.76 |

(14) Intestinal transit is slowed by norepinephrine in a 5-HT-mediated neural pathway.

Intestinal transit during buffer perfusion of the proximal and distal small intestine with intravenous norepinephrine (NE; adrenergic agent) in the presence or absence of the 5-HT receptor antagonist ondansetron was measured, to test the hypothesis that the slowing of intestinal transit also depends on an adrenergic efferent pathway. Perfusion of buffer was through both the duodenal and mid-gut fistulas (2 mL/min over 90 minutes); norepinephrine (0.12 μ g/kg/h) was administered intravenously during the 90 minute perfusion period; and normal saline with or without ondansetron (0.7 mg/kg/h; $n = 2$) was administered in the perfusate to the proximal gut. The results are summarized in Figure 20.

Intestinal transit was slowed by NE so that marker recovery was reduced from 76.9% (Buffer Control in Figure 20) to 13.3% (NE-NS in Figure 20). Ondansetron abolished this slowing effect with marker recovery increased to 63.4% (NE-Ond in Figure 20), to implies that NE (adrenergic efferent) slows transit via a 5-HT-mediated pathway. This result confirms that slowing of intestinal transit is mediated by an adrenergic efferent projecting from the prevertebral ganglion to the gut action on a 5-HT-mediated pathway.

To test the hypothesis that norepinephrine slows intestinal transit via 5-HT₃ receptors, buffer transit across the proximal gut was compared during intravenous administration of norepinephrine with and without lumenally-perfused ondansetron. Five dogs were equipped with duodenal (10 cm from the pylorus) and midgut (160 cm from the pylorus) fistulas as described above. Using occluding Foley catheters, the small intestine was compartmentalized into the proximal (between fistulas) and distal (beyond midgut fistula) one-half of gut. Buffer (pH 7.0) was perfused into the proximal gut at 2 mL/min for 90 min. Intestinal transit of buffer across the proximal gut was compared during intravenous

administration of 50 mg norepinephrine/30 ml/1.5h with and without ondansetron perfused lumenally (0.7 mg/kg/h). Intestinal transit (mean \pm SE) was measured by ^{99m}Tc -DTPA marker recovery in the output of the midgut fistula during the last 30 min of the 90 min experiment. The cumulative % marker recovered was compared using ANOVA and additional analyses by paired t-test.

Results are shown in Table 9 below. These results show that both an adrenergic and serotonergic pathways are involved in the slowing of intestinal transit.

Table 9. Effects of norepinephrine (NE) and ondansetron (Ond) on proximal intestinal transit.

| Transit Across Proximal Gut (Cumulative % Marker Recovered) | |
|--|------------------------------|
| Buffer Control | 68.5 \pm 5.0 ^a |
| Buffer + NE | 16.3 \pm 3.4 ^{ab} |
| Buffer + NE + Ond | 63.0 \pm 4.4 ^b |

^a p < 0.003

^b P < 0.0009

20

(15) The fat-induced jejunal brake depends on the slowing effect of a naloxone-sensitive opioid neural pathway. To test the hypothesis that the slowing of intestinal transit depended on an opioid pathway, the proximal gut was perfused (2 mL/minute for 90 minutes) with buffer containing 60 mM oleate and 0 (normal saline), 3, 6, or 12 mg of naloxone mixed therein, an opioid receptor antagonist. As shown in Figure 21, the fat-induced jejunal brake response depended on the dose of naloxone mixed with the oleate (p<0.05, 1-way ANOVA)(n=7). Specifically, marker recovery was 30.0 \pm 3.6% with 0 mg naloxone, 41.0 \pm 5.2% with 3 mg naloxone, 62.8 \pm 8.2% with 6 mg naloxone and 60.6 \pm 6.1 % with 12 mg naloxone. This result demonstrates that proximal gut fat slows intestinal transit via opioid pathway.

(16) The effect of naloxone was specific for fat-triggered feedback. Intestinal transit was compared during perfusion of the proximal gut with buffer containing 0 (normal saline) or 6 mg naloxone (n=3). The rate of intestinal transit was not significantly affected by the opioid receptor antagonist naloxone when fat was not present in the proximal gut. Marker recovery was $88.0 \pm 1.3\%$ with naloxone and $81.3 \pm 6.1\%$ without naloxone. This implies that the accelerating effect of naloxone was specific for reversing the jejunal brake effect of fat.

(17) The fat-induced ileal brake depends on the slowing effect of an efferent, naloxone-sensitive, opioid neural pathway. The fistulated dog model allowed for the compartmentalization of the afferent limb (distal gut) from efferent limb (proximal gut) of the fat-induced ileal brake. To test for the location of the opioid pathway involved in the slowing of transit by fat, perfusion of buffer was through both the duodenal and mid-gut fistulas (2 mL/min over 90 minutes); the buffer administered through the mid-gut fistula to the distal gut contained 60 mM oleate to induce the ileal brake; 6 mg naloxone was delivered into either the proximal or distal gut (n=11). The results are summarized in Figure 22.

Naloxone delivered to the proximal gut increased marker recovery from $34.6 \pm 4.8\%$ to $76.2 \pm 5.2\%$ (Naloxone in Prox in Figure 21), but naloxone delivered to the distal gut had no effect on the ileal brake (marker recovery of $29.4 \pm 5.4\%$ [Naloxone in Dist in Figure 21]). This result implies that the fat-induced ileal brake depends on an efferent, naloxone-sensitive opioid pathway, because an identical amount of naloxone was delivered into either of the two compartments, but the accelerating effect only occurred when naloxone was delivered into the efferent compartment. Therefore, an opioid pathway is involved that is located peripherally, rather than systemically. The accelerating effect in response to the opioid receptor antagonist is a result of the efferent location of the opioid pathway. It cannot be explained on the basis of chemical interaction with the perfusate, since the acceleration of transit was seen when naloxone was mixed with oleate in Example 14(15), as well as with buffer in this experiment.

(18) Mu and kappa opioid antagonists abolish fat-induced ileal brake. The fat-induced ileal brake (marker recovery 33.1 %) was abolished by a mu antagonist (H2186, Sigma) delivered into the proximal gut so that marker recovery increased to 43.8% at 0.037 mg H2186, 88.2% at 0.05 mg H2186 and 66.8% at 0.1 mg H2186 over 90 minutes. A similar effect was seen

when a kappa antagonist (H3116, Sigma) was used (marker recovery increased to 73.2%% at 0.075 mg H3116, 90.9% at 0.1 mg H3116, and 61.8% at 0.125 mg H3116 over 90 minutes; n= 1).

- 5 (19) Slowing of intestinal transit by distal gut 5-HT depends on a naloxone-sensitive, opioid neural pathway. In Example 14(5), 5-HT in the distal gut slowed intestinal transit, similar to the effect of fat in the distal gut. Since the ileal brake induced by fat in the distal gut was shown to depend on an efferent, naloxone-sensitive opioid pathway (Example 14(17)), it was tested whether the slowing of intestinal transit in response to 5-HT in the distal gut also
10 depends on an efferent, opioid pathway. Buffer was perfused into both the proximal and distal guts at 2 mL/minute for 90 minutes. Either normal saline (Buffer Control in Figure 23) or 5-HT (0.05 mg/kg/h; 5-HT in Dist in Figure 23) was administered to the distal gut over the 90 minute perfusion. When the perfusate to the distal gut contained 5-HT (i.e., 5-HT in Dist), naloxone (6 mg) was simultaneously delivered through the duodenal fistula to the
15 proximal gut over the 90 minutes (Naloxone in Prox in Figure 23). Results are summarized in Figure 23.

First, intestinal transit was slowed by 5HT in the distal gut. Marker recovery was reduced from $79.4 \pm 4.1\%$ (Buffer Control) to $37.0 \pm 1.8\%$ (5-HT in Dist). Second, naloxone in proximal gut abolished this slowing effect with marker recovery increased to
20 $90.1 \pm 4.6\%$ (Naloxone in Prox). These results imply that slowed intestinal transit in response to 5-HT in the distal gut, depends on an efferent opioid pathway.

The foregoing examples being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.

CLAIMS

1. A method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, said method comprising:

detecting in the subject by suitable detection means, the presence of SIBO, wherein a population of proliferating bacteria is present in the small intestine of the subject, or detecting with said means the absence of SIBO; and, if the presence of SIBO is detected in the subject,

depriving the bacterial population of nutrient(s) sufficiently to inhibit the growth of said bacteria in the small intestine, and thereby at least partially eradicating SIBO in the human subject.

2. The method of Claim 1, wherein the SIBO-caused condition is selected from the group consisting of irritable bowel syndrome, fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, depression, impaired mentation, impaired memory, halitosis, tinnitus, sugar craving, autism, attention deficit/hyperactivity disorder, drug sensitivity, an autoimmune disease, and Crohn's disease.

3. The method of Claim 2, wherein the autoimmune disease is systemic lupus erythematosus or multiple sclerosis.

4. The method of Claim 1, further comprising:
in the presence of SIBO in the human subject,
causing the subject to consume, for a sustained period, a diet consisting essentially of nutrients that upon arrival in the upper gastrointestinal tract of the subject, are at least partially predigested, said sustained period being sufficient to at least partially eradicate SIBO in the human subject.

5. The method of Claim 4, wherein the period is at least about three days.

6. The method of Claim 4, wherein the at least partially predigested nutrients are contained in a commestible total enteral nutrition formulation.

7. The method of Claim 4, further comprising:
administering to the subject a pancreatic enzyme supplement before or substantially simultaneously with a meal, such that nutrients contained in said meal are at least partially predigested in the upper gastrointestinal tract of the subject by the activity of said pancreatic enzyme supplement.

8. The method of Claim 1, further comprising:

in the presence of SIBO in the human subject,

enhancing the digestion and/or absorption of the nutrient(s) in the upper gastrointestinal tract of said human subject by slowing transit of said nutrients across the upper gastrointestinal tract of said human subject, thereby at least partially depriving the bacterial population of the nutrient(s).

9. The method of Claim 8, further comprising:

administering a pharmaceutically acceptable composition to said human subject by an oral or enteral delivery route, said human subject having an intrinsic cholinergic afferent neural pathway projecting from a peptide YY-sensitive primary sensory neuron in the intestinal wall of said subject to a prevertebral celiac ganglion and having an adrenergic efferent neural pathway projecting from said ganglion to one or more enterochromaffin cells in the intestinal mucosa and/or to a serotonergic interneuron linked in a myenteric plexus and/or submucous plexus to an opioid interneuron, said opioid interneuron also being linked by an intestino-fugal opioid pathway projecting to said ganglion, with one or more neural connections to the central nervous system and back to the gut projecting from the ganglion,

said pharmaceutically acceptable composition comprising an active agent, said active agent being selected from the group consisting of

- (A) active lipids;
- (B) serotonin, serotonin agonists, or serotonin re-uptake inhibitors;
- (C) peptide YY or peptide YY functional analogs;
- (D) calcitonin gene-related peptide or functional analogs thereof;
- (E) adrenergic agonist;
- (F) opioid agonists;
- (G) combinations of any of (A), (B), (C), (D), (E) and/or (F); and
- (H) antagonists of receptors for any of (B), (C), (D), (E) and/or (F),

said active agent being delivered in an amount and under conditions such that the cholinergic intestino-fugal pathway, at least one prevertebral ganglionic pathway, the adrenergic efferent neural pathway, the serotonergic interneuron and/or the opioid interneuron are activated by the action of any of (A) through (G), whereby the rate of upper gastrointestinal transit in the subject is slowed, thereby enhancing the digestion and/or absorption of the nutrient(s) in the upper gastrointestinal tract of said human subject.

10. The method of Claim 8, further comprising:

administering a gastrointestinal transit-slowing composition comprising a carrier and a dispersion consisting essentially of an active lipid in the carrier, the active lipid being selected from

the group consisting of saturated and unsaturated fatty acids, fully hydrolyzed fats and mixtures thereof, in an amount and in a form effective to promote contact of the lipid with the subject's small intestine and thereby slow gastrointestinal transit and at least partially eradicate SIBO in the human subject.

11. The method of Claim 10, wherein the active lipid is selected from the group consisting of:

(A) caprolic acid, caprylic acid, capric acid, lauric acid, myristic acid, oleic acid, palmitic acid, stearic acid, palmitoleic acid, linoleic acid, linolenic acid, trans-hexadecanoic acid, elaidic acid, columbinic acid, arachidic acid, behenic acid eicosenoic acid, erucic acid, brassidic acid, cetoleic acid, nervonic acid, Mead acid, arachidonic acid, timnodonic acid, clupanodonic acid, or docosahexaenoic acid;

(B) pharmaceutically acceptable salts of any of (A); and

(C) mixtures of any of (A) or (B).

10

12. The method of Claim 11, wherein the active lipid comprises oleic acid or a pharmaceutically acceptable oleate salt.

13. The method of Claim 10, wherein the active lipid comprises fully hydrolyzed fats.

14. The method of Claim 10, wherein the active lipid comprises a fatty acid or a pharmaceutically acceptable salt thereof.

15. The method of Claim 10, wherein the active lipid is:

(A) a fatty acid selected from the group of (C4-C24) saturated and unsaturated fatty acids;

(B) a pharmaceutically acceptable salt of any of (A); or

5 (C) a mixture of any of (A) and/or (B).

16. The method of Claim 10, wherein the fatty acid comprises oleic acid, a pharmaceutically acceptable oleate salt, or a mixture of either of these with other fatty acids or salts thereof.

17. The method of Claim 9, wherein oral administration is by ingestion of coated or uncoated microspheres or particles, of a dispersible powder or granule formulation, of a

suspension, emulsion, solution, syrup, or elixir, or of a coated or uncoated tablet, troche, capsule, caplet, or lozenge.

18. The method of Claim 10, wherein oral administration is by ingestion of coated or uncoated microspheres or particles, of a dispersible powder or granule formulation, of a suspension, emulsion, solution, syrup, or elixir, or of a coated or uncoated tablet, troche, capsule, caplet, or lozenge.

19. The method of Claim 9, wherein the active agent is selected from the group consisting of serotonin, serotonin agonists, serotonin re-uptake inhibitors, 5-HT₃ receptor antagonists, and 5-HT₄ receptor antagonists.

20. The method of Claim 19, wherein the active agent is serotonin, and the serotonin is administered to the human subject, before or substantially simultaneously with a meal, in an amount from about 0.03 to about 0.1 mg/kg body mass.

21. A method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, said method comprising:

detecting in the subject by suitable detection means, the presence of SIBO, wherein a population of proliferating bacteria is present in the small intestine of the subject, or detecting with said means the absence of SIBO; and, if the presence of SIBO is detected in the subject,

introducing into the lumen of the small intestine of the subject a pharmaceutically acceptable disinfectant composition in an amount sufficient to inhibit the growth of said bacteria in the small intestine, and thereby at least partially eradicating SIBO in the human subject.

22. The method of Claim 21, wherein the pharmaceutically acceptable disinfectant composition consists essentially of

- (A) hydrogen peroxide,
- (B) a bismuth-containing compound,
- (C) an iodine-containing compound, or
- (D) a salt of (B) or (C).

23. The method of Claim 21, wherein the SIBO-caused condition is selected from the the group consisting of irritable bowel syndrome, fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, depression, impaired mentation, impaired memory, halitosis, tinnitus, sugar craving, autism, attention deficit/hyperactivity disorder, drug sensitivity, an autoimmune disease, and Crohn's disease.

24. The method of Claim 23, wherein the autoimmune disease is systemic lupus erythematosus or multiple sclerosis.

25. A method of treating small intestinal bacterial overgrowth (SIBO) or a SIBO-caused condition in a human subject, said method comprising:

detecting in the subject by suitable detection means, the presence of SIBO, wherein a population of proliferating bacteria is present in the small intestine of the subject, or detecting with
5 said means the absence of SIBO; and, if the presence of SIBO is detected in the subject,

administering to the subject a pharmaceutically acceptable composition comprising a stabilizer of mast cell membranes in the luminal wall of the small intestine, in an amount sufficient to inhibit a mast cell-mediated immune response in the human subject.

26. The method of Claim 25, wherein the SIBO-caused condition is selected from the the group consisting of fibromyalgia, chronic pelvic pain syndrome, chronic fatigue syndrome, depression, impaired mentation, impaired memory, halitosis, tinnitus, sugar craving, autism, attention deficit/hyperactivity disorder, drug sensitivity, an autoimmune disease, and Crohn's disease.

27. The method of Claim 26, wherein the autoimmune disease is systemic lupus erythematosus or multiple sclerosis.

28. The method of Claim 25, wherein the stabilizer of luminal mast cells is oxatamide or chromoglycate.

29. A method of screening for the abnormally likely presence of SIBO in a human subject, comprising:

obtaining a serum sample from the subject;
quantitatively determining a concentration in the serum sample of serotonin, one or more
5 unconjugated bile acid(s), and/or folate, an abnormally elevated serum concentration of one or more of these being indicative of a higher than normal probability that SIBO is present in the subject.

30. A method of detecting small intestinal bacterial overgrowth (SIBO) in a human subject, comprising:

detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by said human subject, after said human subject has ingested a

- 5 controlled quantity of a substrate, said gas mixture being at least partially produced by the intestinal microflora of said human subject.

31. The method of Claim 30, wherein the substrate is an isotope-labeled sugar or a sugar that is poorly digestible by a human.

32. The method of Claim 31, wherein the sugar is glucose, lactose, sucrose, lactulose or xylose.

33. The method of Claim 30, wherein detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in the exhaled gas mixture is accomplished by gas chromatography and/or a radiation detection system.

34. The method of Claim 30, wherein detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in the exhaled gas mixture is accomplished by mass spectrometry.

35. The method of Claim 30, wherein detecting the relative amounts of methane, hydrogen, and at least one sulfur-containing gas in the exhaled gas mixture is accomplished using thin-layer chromatography, high pressure liquid chromatography, an electrochemical voltametric sensor, or a polarographic cell

36. The method of Claim 30, wherein the at least one sulfur-containing gas is hydrogen sulfide or a sulfhydryl compound.

37. The method of Claim 30, wherein the at least one sulfur-containing gas is methanethiol, dimethylsulfide, dimethyl disulfide, an allyl methyl sulfide, an allyl methyl sulfide, an allyl methyl disulfide, an allyl disulfide, an allyl mercaptan, or a methylmercaptan.

38. A method of determining the relative severity of SIBO or a SIBO-caused condition in a human subject in whom SIBO has been detected, comprising:

detecting in the subject by suitable detection means, the presence of SIBO, or detecting with said means the absence of SIBO; and, if the presence of SIBO is detected in the subject,

- 5 detecting in the subject by suitable detection means a relative level of intestinal permeability, abnormally high intestinal permeability indicating a relatively severe SIBO or SIBO-caused condition in the subject.

39. A kit for the diagnosis of SIBO or a SIBO-caused condition, comprising:
at least one breath sampling container, a pre-measured amount of a substrate, and instructions for
a user in detecting the presence or absence of SIBO by determining the relative amounts of methane,
hydrogen, and at least one sulfur-containing gas in a gas mixture exhaled by said human subject,
5 after said human subject has ingested a controlled quantity of the substrate.

40. The kit of Claim 39, wherein the pre-measured substrate is isotope-labeled
or poorly digestible by a human.

41. The kit of Claim 39, wherein the pre-measured substrate is glucose, lactose,
sucrose, lactulose or xylose.

42. The kit of Claim 39, wherein the pre-measured substrate is a sugar.

43. The kit of Claim 39, further comprising standardized samples of methane,
hydrogen, and at least one sulfur-containing gas.

44. The kit of Claim 39, wherein the at least one sulfur-containing gas is
hydrogen sulfide or a sulphydryl compound.

45. The kit of Claim 39, wherein the at least one sulfur-containing gas is
methanethiol, dimethylsulfide, dimethyl disulfide, an allyl methyl sulfide, an allyl methyl sulfide,
an allyl methyl disulfide, an allyl disulfide, an allyl mercaptan, or a methylmercaptan.

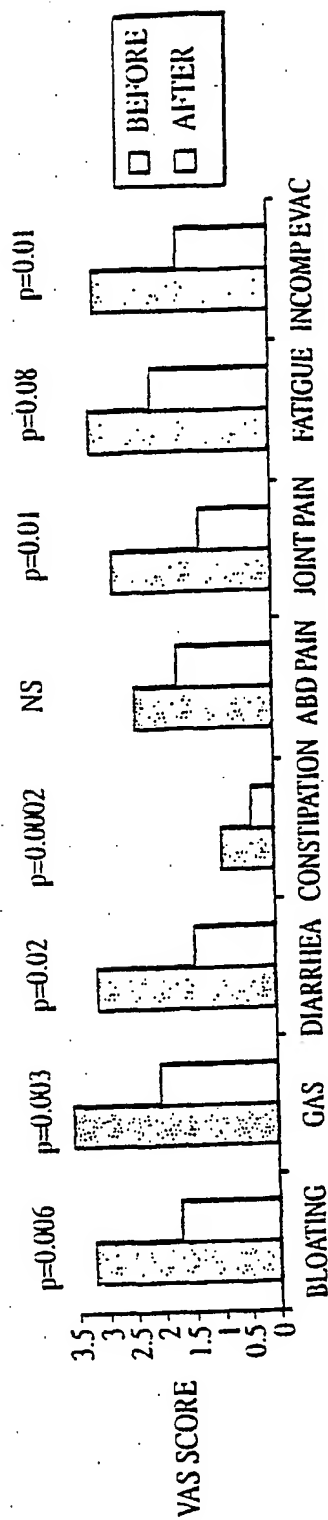


FIG. 1

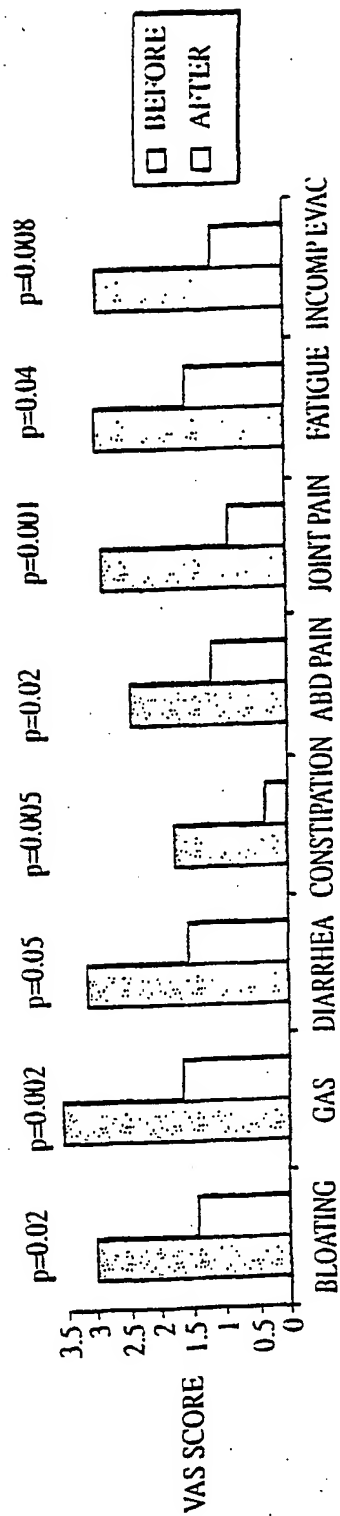


FIG. 2

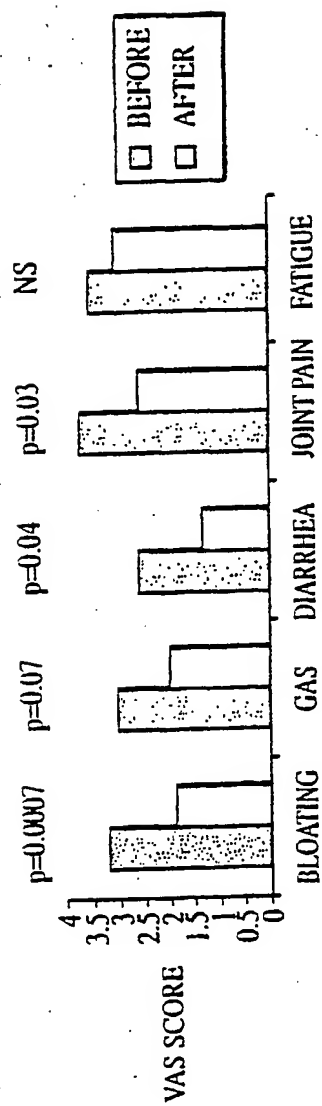


FIG. 3

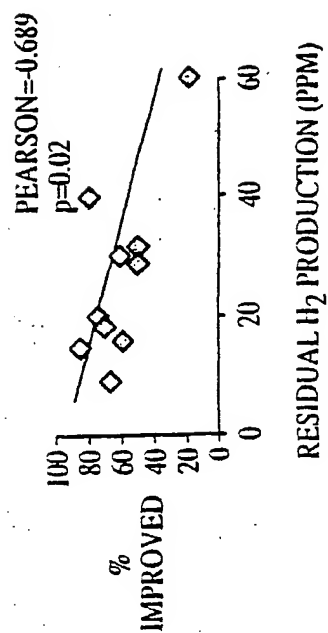


FIG. 4

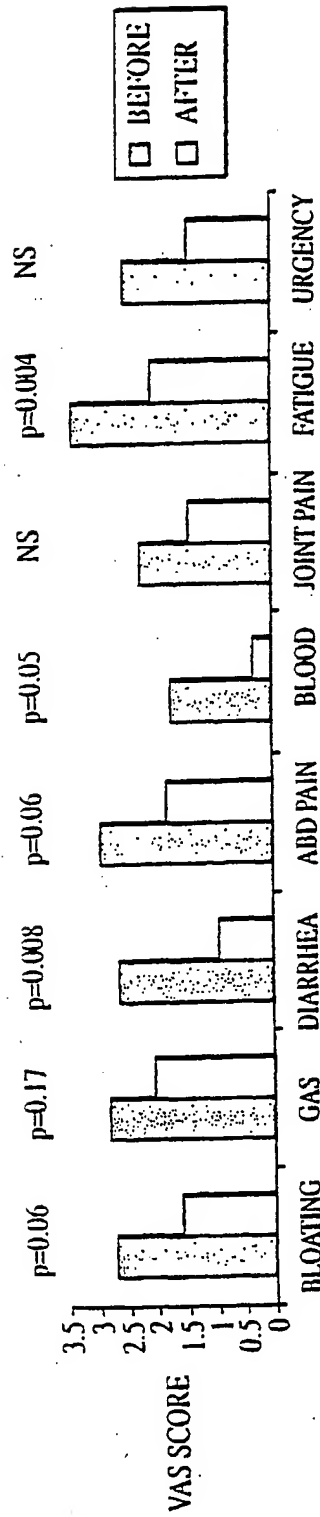


FIG. 5

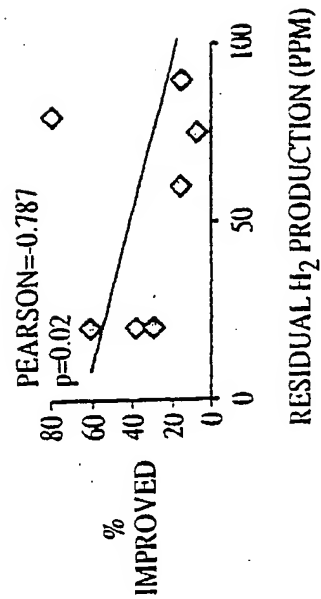


FIG. 6

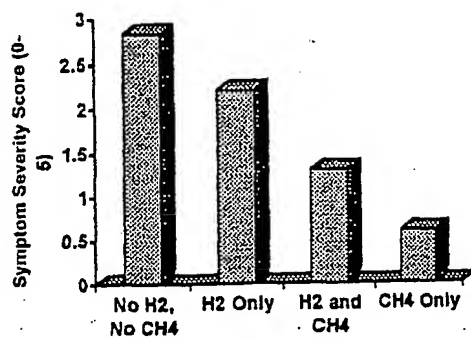


Figure 7

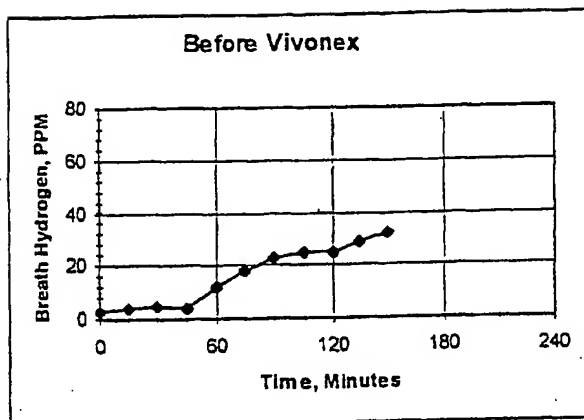


Figure 8A

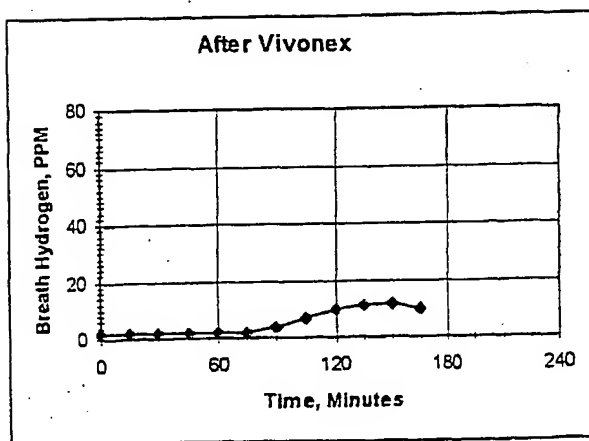


Figure 8B

Figure 8

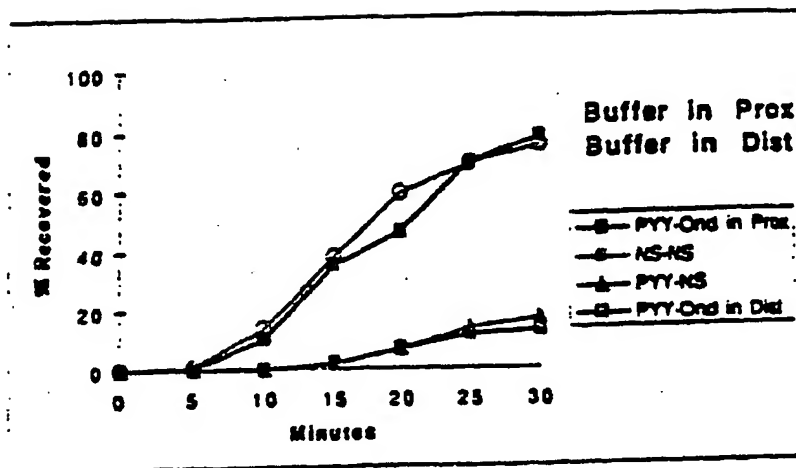


Figure 9

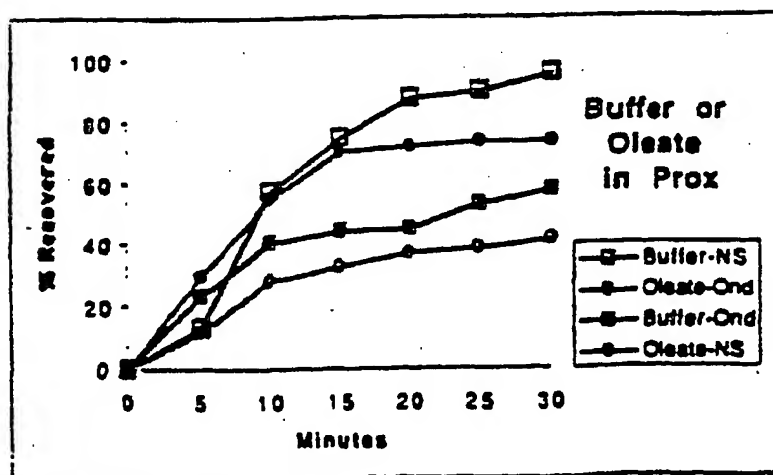


Figure 10

7/13

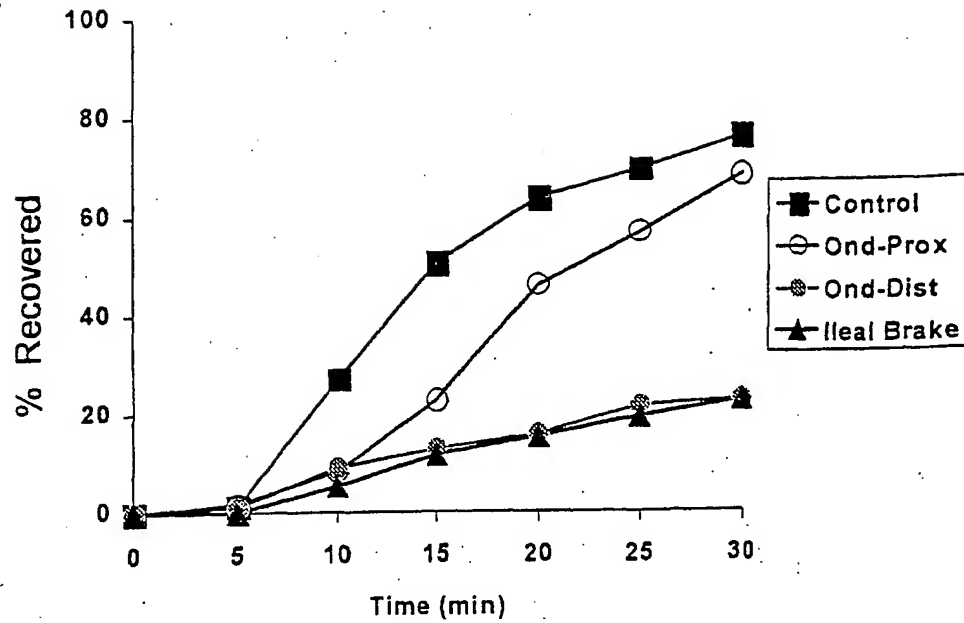


Figure 11

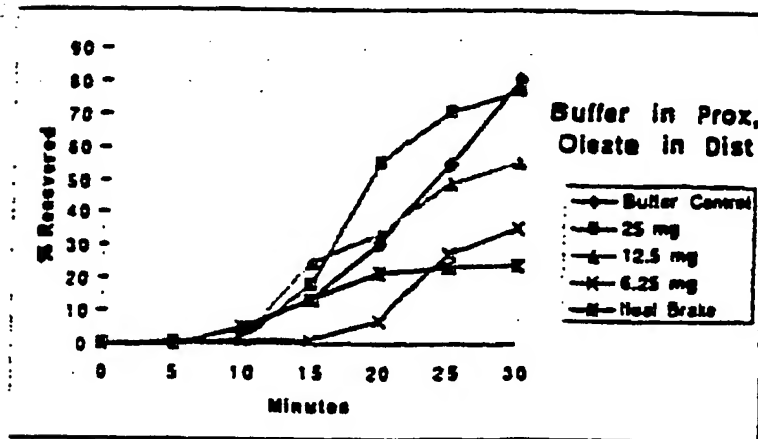


Figure 12

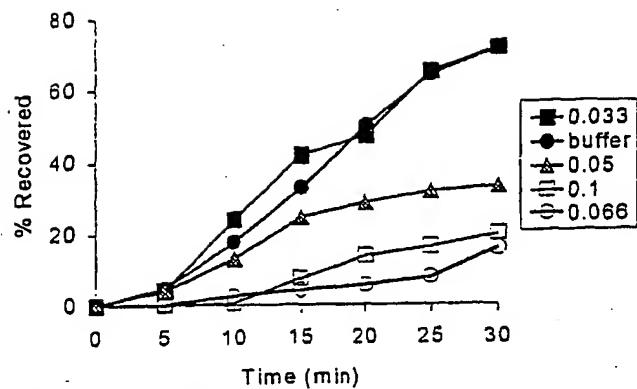
Dose-response: $p < 0.00001$

Figure 15

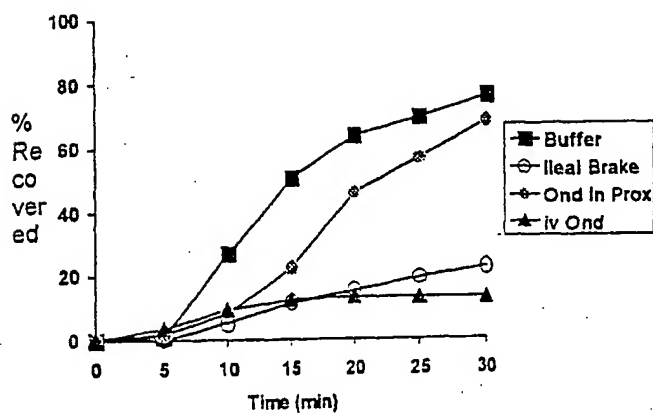


Figure 13

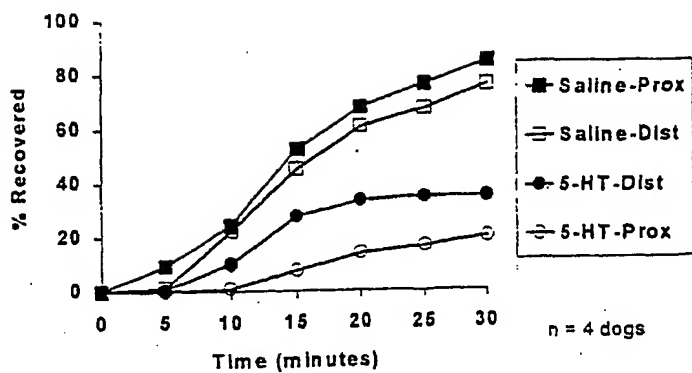


Figure 16

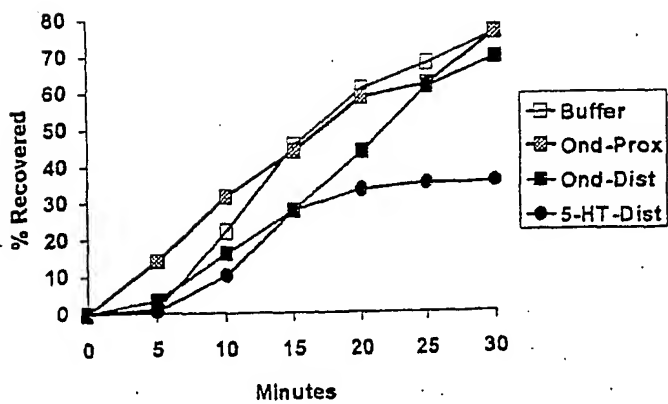
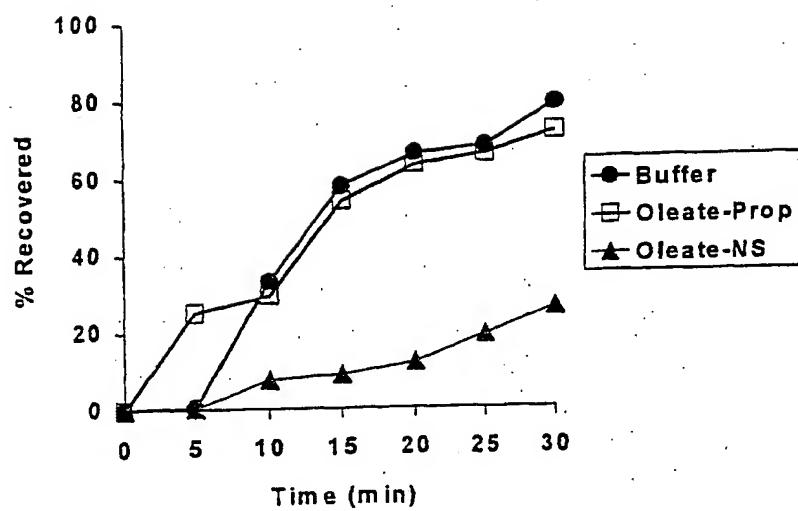


Figure 14

10/13

*Figure 17*

11/13

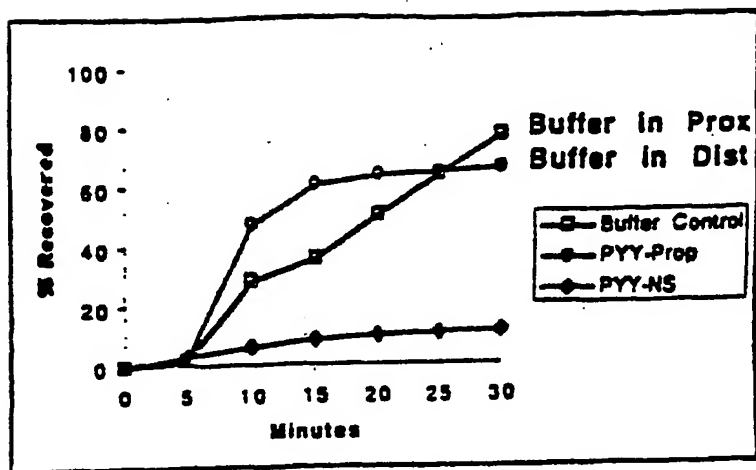


Figure 18

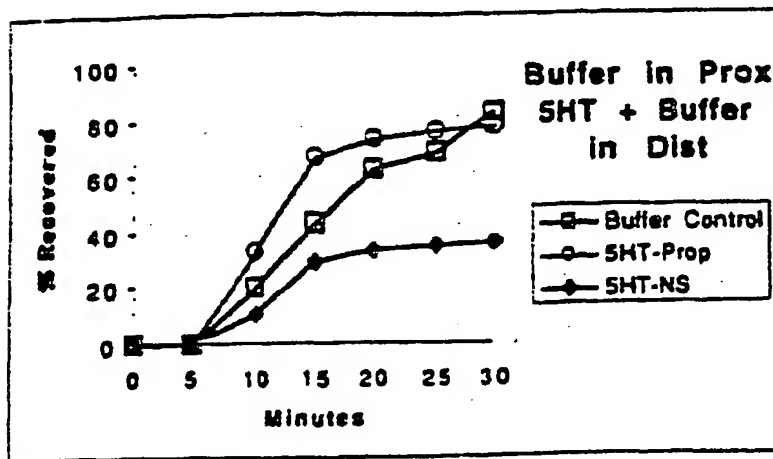


Figure 19

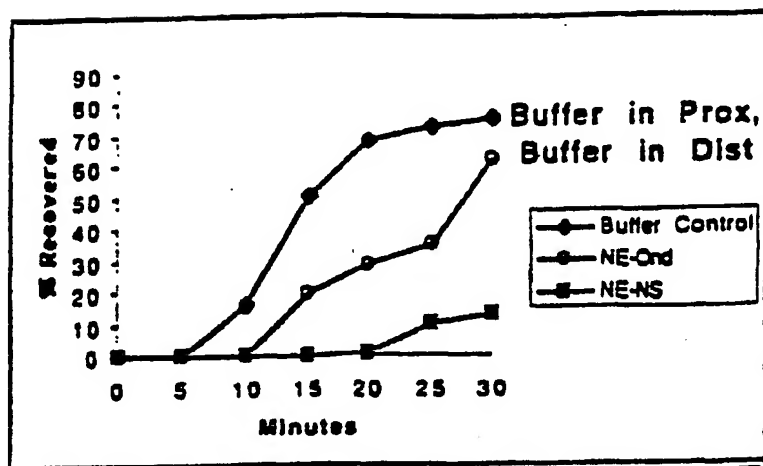


Figure 20

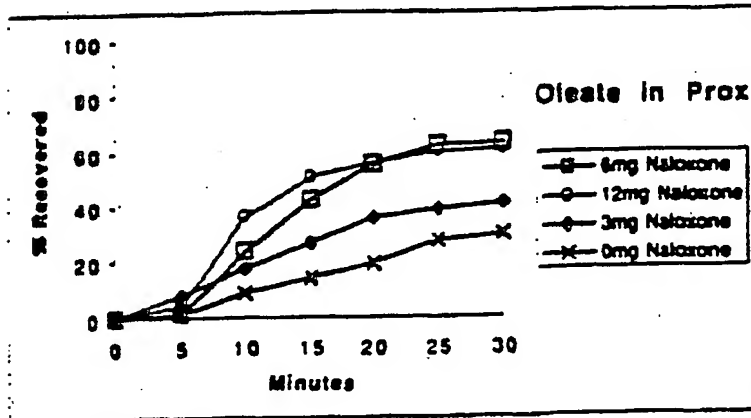
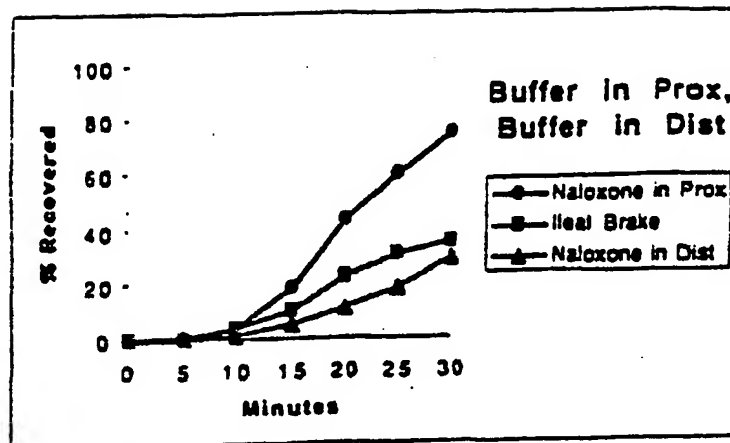
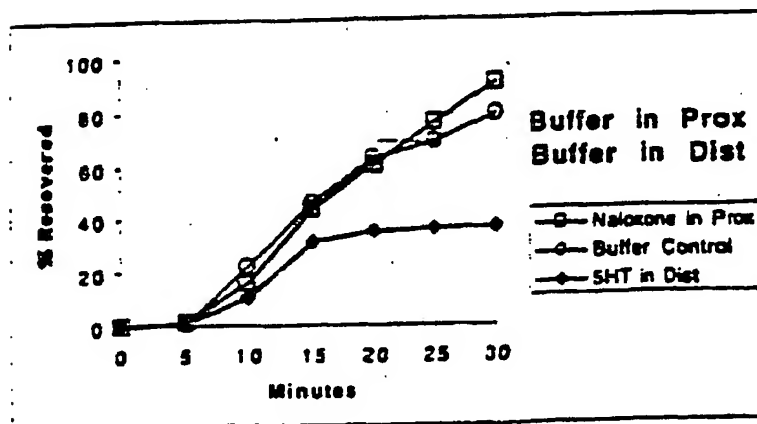


Figure 21

*Figure 22**Figure 23*